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ACCIDENT SURVEYS AND SAFETY PROGRAMS IN TWO HOSPITAL CLINICAL LABORATORIES*

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INTRODUCTION

One area of concern in the profession of medical technology is safety and accident prevention for the professional and non-professional personnel and for the student worker. The medical technologist in the clinical laboratories has turned to the established safety measures used in industry and hospitals for guidance in the planning of a suitable safety program for the clinical laboratory situation.[8,9,10,12,13] Little has been done to evaluate the efficiency of the safety programs in the clinical laboratories. The main purpose of this paper is to provide an analysis of the safety programs of two hospital laboratories by statistical and epidemiological methods, comparing these hospitals with one another and with reported accident statistics in hospitals and industry.

The major emphasis in the past and the statistics published have been frequency rates of disabling accidents. It is only recently that there have been proposals for the review of *all* accidents incurred to determine the effectiveness of safety programs.[3]

METHOD

The following formulae have been used in the calculations of the data herein presented:[3]

1. Total accident frequency rate

$$\frac{\text{Number of accidents} \times 100{,}000}{\text{Number of man hours of exposure}} = \text{Total accident frequency rate/100,000 hours}$$

2. Disabling injury frequency rate

$$\frac{\text{Number of disabling accidents} \times 1{,}000{,}000}{\text{Number of man hours of exposure}} = \text{Injury frequency rate}$$

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3. Severity rate

$$\frac{\text{Total number of days lost} \times 1{,}000{,}000}{\text{Number of man hours of exposure}} = \text{Severity rate}$$

4. Equivalent hours

One person @ 40 hours/week = 2080 equivalent hours/year/full time employee

In the computation of the severity rate, it must be remembered to check the charge ranges for permanent total or partial disabilities. For example, the scheduled charge for the loss of the distal phalange of an index finger would be 100 days even though the employee might return to work before this time had elapsed.[3] Since there were no such injuries in this study, it was decided not to include this discussion within this paper.

RESULTS

The following tables 1-9 show the total accident frequency rates in the laboratory of a University Hospital and in the laboratory of a Private Hospital during the four year period 1956-1959. Accidents causing disabling injuries have been included in these statistics. The data for the tables has been obtained from the laboratory accident reports of a University Hospital and a Private Hospital.

DISCUSSION

The documentation of the total accident frequency rate of two hospitals is intended to help other clinical laboratories compare and evaluate their safety programs. In discussing total accident frequency rates and reviewing the yearly figures, it is important to bear in mind that high frequency rates do not necessarily mean poor safety programs, but can give an indication of base line safety operation for a particular laboratory.[3] This base line of safety operation then can be utilized for evaluation from year to year as a basis for necessary changes.

The total frequency rate for clinical laboratories having fewer personnel and, therefore, fewer number of man hours of exposure will be greatly affected by any increase in accidents over zero. It is evident that in laboratories having fewer personnel one accident can skew the total accident frequency rate to a point where it might appear to be an unsafe laboratory. For example, in Table 1 in 1959 a University Hospital had a total of 9 accidents to Medical Technologists with a total accident frequency rate of 6.4 whereas in Table 3 in 1957 a Private Hospital had a total of 1 accident to a Medical Laboratory Assistant with a total accident frequency rate of 48.1.

TABLE 1—Total Accident Frequency Rate
Staff Medical Technologists Comparison Study
1956 - 1959

Year	Medical Technologist		Equivalent Hours		Total Number of Accidents to Medical Technologists		Number of Accidents/100,000 Man Hours	
	University Hospital	Private Hospital	University Hospital	Private Hospital	University Hospital	Private Hospital	University Hospital	Private Hospital
1956	53.5	21.0	111,280	43,680	3	15	2.7	34.3
1957	58.5	22.0	121,680	45,760	3	12	2.5	26.2
1958	62.0	23.5	128,960	48,880	8	3	6.2	6.1
1959	68.0	25.5	141,440	53,040	9	14	6.4	26.4
Total	...	...	503,360	191,360	23	44	..	...
Average Frequency Rates							4.6	23.0

TABLE 2—Total Accident Frequency Rate
Students in Clinical Laboratories Comparison Study
1956 - 1959

Year	Senior Students in Medical Technology	Medical Laboratory Assistant Students	Equivalent Hours		Total Number of Accidents to Students		Number of Accidents/100,000 Man Hours	
	University Hospital	Private Hospital	University Hospital	Private Hospital	University Hospital	Private Hospital	University Hospital	Private Hospital
1956	15	1.5	31,200	3,120	3	0	9.6	0
1957	19	1.0	39,520	2,080	6	0	15.2	0
1958	23	0.5	47,840	1,040	13	0	27.2	0
1959	28	1.0	58,240	2,080	14	0	24.0	0
Total	..	..	176,800	8,320	36	0	...	..
Average Frequency Rates							20.4	0

TABLE 3—Total Accident Frequency Rate
Staff Medical Laboratory Assistants Comparison Study
1956 - 1959

Year	Staff Medical Laboratory Assistants		Equivalent Hours		Total No. Accidents to Staff Medical Laboratory Assistants		Number of Accidents/100,000 Man Hours	
	University Hospital	Private Hospital	University Hospital	Private Hospital	University Hospital	Private Hospital	University Hospital	Private Hospital
1956	0	1	....	2,080	..	0	..	0
1957	0	1	....	2,080	..	1	..	48.1
1958	0	1	....	2,080	..	0	..	0
1959	0.5	1	1,040	2,080	0	0	0	0
Total	..	..	1,040	8,320	0	1	..	...
Average Frequency Rate							0	12.0

TABLE 4—Total Accident Frequency Rate
Laboratory Attendant (Non-Professional) Comparison Study
1956 - 1959

Year	Laboratory Attendant		Equivalent Hours		Total No. Accidents to Laboratory Attendants		Number of Accidents/100,000 Man Hours	
	University Hospital	Private Hospital	University Hospital	Private Hospital	University Hospital	Private Hospital	University Hospital	Private Hospital
1956	5.0	4.2	10,400	8,736	0	2	0	22.9
1957	7.5	4.2	15,600	8,736	2	4	12.8	45.8
1958	10.0	4.2	20,800	8,736	0	3	0	34.3
1959	13.0	4.2	27,040	8,736	2	3	7.4	34.3
Total	...	..	73,840	34,944	4	12	...	...
Average Frequency Rate							5.4	34.3

TABLE 5—Total Accident Frequency Rate
All Laboratory Workers—Comparison Study
1956 - 1959

Year	All Laboratory Workers		Total Equivalent Hours		Total Number of Accidents to All Laboratory Workers		Number of Accidents/100,000 Man Hours	
	University Hospital	Private Hospital	University Hospital	Private Hospital	University Hospital	Private Hospital	University Hospital	Private Hospital
1956	73.5	27.7	152,880	57,616	6	17	3.9	29.5
1957	85.0	28.2	176,800	58,656	11	17	6.2	29.0
1958	95.0	29.2	197,600	60,736	21	6	10.6	9.9
1959	109.5	31.7	227,760	65,936	25	17	11.0	25.8
Total	....	...	755,040	242,944	63	57	....	...
Average Frequency Rate							8.3	23.5

Table 6 shows that the variability of institutional programs with regard to the reporting of accidents tends to create a misleading picture. The scrupulous reporting of minor injuries such as serum or spinal fluid over-pipetting accidents or paper cut accidents which did not cause disabling injuries shows that all employees were being urged to report even the most minor accidents without regard to competition.

The principal cause of all accidents in these two hospitals during the four-year period was cuts by glass. All laboratory workers recognize the potential hazard of glass, but it is apparent that more emphasis needs to be placed on safety measures regarding this material. Differences in the accident frequency rates in laboratories will be weighted by the type of worker as can be shown by the fact that most of the accidents caused by over-pipetting into the mouth occurred in the student group. The number of accidents caused by microtome knives differed greatly in the two laboratories. In evaluating the percent of accidents caused by microtome knives, the working conditions of the laboratory must be considered as well as the safety practices of the personnel.

TABLE 6—Percentage Comparison of Causes of Accidents in the Clinical Laboratory

Causative Agent	University Hospital		Private Hospital	
	Number	Percent	Number	Percent
Glass	25	39.7	19	33.3
Acid or alkali	16	25.4	0	0.0
Serum or spinal fluid over-pipetting	9	14.3	0	0.0
Blades	4	6.3	7	12.3
Microtome	2	3.2	14	24.6
Scissors	0	0.0	2	3.5
Paper	0	0.0	2	3.5
Formaldehyde	0	0.0	1	1.7
Needles	0	0.0	3	5.3
Bruise	7	11.1	3	5.3
Burn	0	0.0	6	10.5
Total	63	100.0	57	100.0

Comparison figures in Table 7 for areas of the body injured showed that the greatest percentage of accidents occurred to the hands, arms and fingers. The mouth as a specific part of the body is usually not considered in over-all hospital reporting; however, the mouth was considered because of the possible hazard of infectious hepatitis, other infectious diseases, and burns by chemical reagents.

TABLE 7—Comparison of Areas of Body Injured 1956 - 1959

Area Injured	University Hospital		Private Hospital	
	Number	Percent	Number	Percent
Hands, arms and fingers	29	46.0	52	91.2
Mouth	19	30.1	0	0.0
Eyes	6	9.5	1	1.7
Other	9	14.3	4	7.0
Total	63	99.9	57	99.9

The next area of consideration is the injury frequency rate for disabling accidents or "how serious were those reported injuries: did they disable the employee?"[1] The average disabling injury frequency rate has been reported as 8.6/1,000,000 man hours for all types of hospitals and 4.5/1,000,000 man hours for clinical laboratories (Claque, 1956).[2,6] To provide a broader base line for comparison, the following statistics are cited:

Accident Frequency Rates for Disabling Injuries in Industry[7]

All industries	6.17/1,000,000 man hours
Automobile manufacturing	2.44/1,000,000 man hours
Chemical manufacturing	3.56/1,000,000 man hours
Glass manufacturing	4.29/1,000,000 man hours
Construction	16.99/1,000,000 man hours
Coal mining	27.39/1,000,000 man hours

It is evident from Table 8 that the disabling accident frequency rate of the clinical laboratories in this survey fall below the disabling accident frequency rate of 6.17/1,000,000 man hours for all industry, the frequency rate of 8.6/1,000,000 man hours for all hospitals, and the frequency rate of 4.5/1,000,000 man hours for clinical laboratories.

In order to evaluate the difference in frequency rates between these two hospitals, the severity of the accident must be considered. The severity rates for the four-year period reviewed were very comparable, but the possibility of any one accident causing a long term disability is great in any laboratory. The average disabling injury frequency rate and the average severity rate for each hospital laboratory over this four-year period are unusual in that they are the same. The total number of days lost by the personnel involved in the accidents was, in each laboratory, equal to the total number of accidents for the period; therefore, these rates were the same. Had any one of these accidents caused a prolonged loss of time, the severity rate would have been markedly increased.

TABLE 8—Injury Frequency Rate
All Laboratory Workers Comparison Study
1956 - 1959

Total Equivalent Hours/4 Years		Total Number Disabling Accidents/4 Years		Injury Frequency Rate	
University Hospital	Private Hospital	University Hospital	Private Hospital	University Hospital	Private Hospital
755,040	242,944	3	1	4.0	4.1

TABLE 9—Severity Rate
All Laboratory Workers Comparison Study
1956 - 1959

Total Equivalent Hours/4 Years		Total Number Days Lost		Severity Rate	
University Hospital	Private Hospital	University Hospital	Private Hospital	University Hospital	Private Hospital
755,040	242,944	3	1	4.0	4.1

Since all accidents are caused by unsafe acts or unsafe conditions, and since it is estimated, according to Heinrich, that 98% of all accidents are preventable,[4] hospital workers should have a greater obligation to review accident reports and analyze them so that efforts can be directed toward preventing their recurrence. The most effective method of providing this prevention and protection is to have a well-defined and

working safety program. General safety rules are provided by all hospital administrations, but it is the responsibility of all laboratory supervisors to augment these regulations by additional specific regulations pertaining to the laboratory areas. The following recommendations encompass the essential features of the safety programs of the two laboratories.

Safety Program:

The first step toward a sound safety program should be the formation of a laboratory safety committee. The chairman of this committee should be appointed to the hospital safety committee as a representative of the laboratory. This individual will provide liaison between these two committees. The laboratory committee may vary in size depending upon the organizational structure of the laboratory service. Committee members should be chosen on the basis of their interest in safety and their leadership ability. In larger laboratories, it may be advisable to have the membership include one representative from each division of the laboratory and one representative from each worker classification. The chairman should be responsible for compiling duplicate copies of the original accident reports of laboratory personnel. Meetings should be held once a month and all laboratory accidents which occurred during the preceding month should be analyzed at this time. It may be advantageous to post the minutes of this meeting and a summary of the accident statistics. The base line of safety operation can be utilized on a monthly or on a yearly basis. A chart or graph may stimulate all workers to strive for a greater measure of laboratory safety. This committee should be given authority to make recommendations for the purchase of safety devices and physical changes that are needed for safety within the laboratory area. One of the chief functions of this committee should be the formulation of safety recommendations.[5] The following suggestions do not begin to include all the danger areas but should provide an initial "check list."

Safety Check List:[10,11]

1. All electrical receptacles should be of explosion proof type, no more than one plug per receptacle, and no extension cords used at any time.
2. Chemical masks, safety goggles, safety gloves, etc., should be available and used whenever the procedure requires it.
3. Adequate protection should be given whenever applying stress to glass.
4. All laboratory glassware that is cracked or chipped should be discarded.
5. Pressure sensitive tape labels should be used if at all possible; if not, a sponge should be used to moisten labels for laboratory samples and specimens.
6. A "Flammable Liquids Room" should be built either adjacent to the laboratory or in connection with Pharmacy or Central Service for the storage of all flammable liquids, except for a small daily supply. All flammable liquids should be stored in polyethylene or Underwriters Laboratories approved safety containers. All such containers should be properly labeled.

7. Refrigerators used to store flammable liquids should have the light bulb and socket removed before such use.
8. "No Smoking" regulations should be enforced in the laboratory departments and corridors adjoining the laboratory.
9. All reagent bottles should be marked legibly; caution labels should be used on all containers with hazardous material.
10. All bacteriology incubators, refrigerators and cupboards used for culture incubation should be locked whenever possible.
11. All bacteriology pipettes should be cotton plugged, or similarly protected, for safety in transferring specimens.
12. Separate disposal cans should be easily available and labeled for glass, contaminated material, laundry, and uncontaminated waste.
13. Strong chemicals and toxic solvents should always be pipetted with suction.
14. Fume hoods should be used whenever dispensing acids, strong alkalis or materials with toxic or poisonous fumes.
15. All gas cylinders should be chained or placed on dollies.
16. Microtomes should never be left unattended while any of the knife screws or clamp screws are loosened.
17. Microtome knives should be removed from the holder before the microtomes are cleaned.
18. All disposable needles, blades and syringes should be autoclaved before discarding. After sterilization, these can be discarded by incineration or discarded with the broken glassware.
19. The proper restraining equipment should be used when working with all animals.
20. All acetone or ether fires should be fought with the chemical extinguishers unless the CO_2 extinguisher is needed for individual safety.
21. Safety step ladders should be used.
22. An eye cup should be available for immediate use. A water faucet with eye attachment should be included in the plans for new or remodeled laboratories.
23. A safety shower should be included in all plans for new or remodeled laboratories.

SUMMARY

Statistical and epidemiological methods were used to compare the safety programs of two hospital clinical laboratories.

1. The total accident frequency rate, disabling accident frequency rate, and severity rate have been determined for each hospital and for each of four classifications of laboratory workers: a. the staff Medical Technologist, b. student Medical Technologist and student Medical Laboratory Assistant, c. staff Medical Laboratory Assistant, d. non-professional laboratory attendant. These statistics have been presented to encourage other clinical laboratories to formulate their own base line of safety operation for the evaluation of their safety programs.

2. A survey of the causes of accidents and the areas of the body injured has been documented and discussed.

3. The disabling accident frequency rate for each laboratory has been compared with the average frequency rates of industry, the average

frequency rate of all hospitals, and the average frequency rate for clinical laboratories.

4. A suggested safety program and a safety check list have been presented to aid in the organization of an active laboratory safety program.

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IMPROVING SENSITIVITY TESTS BY ADDING TRIPHENYLTETRAZOLIUM*

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In many cases it is difficult to read disc inhibition zones on an agar plate. From a laboratory point of view it would be desirable if some method could be devised to make sensitivity plates easier to read. Kelley and Fulton (3) used 2,3,5 triphenyltetrazolium chloride (TTC) in motility medium to make the spreading movement of the bacterial growth easier to detect. Canizares and Shatin (2) used this same salt in their studies of dermatophytes. This compound forms a colorless aqueous solution. When reduced, a compound is formed which is insoluble in water but soluble in alcohol and acetone. The dark red substance is called triphenylformazan. Most of the bacteria dealt with in clinical laboratories incorporate TTC in their metabolism, reducing the salt to the red formazan (1), which is deposited as visible red granules on the cells. The essential reaction is:

$$\text{Triphenyltetrazolium} + 2e + 2H^{+} \rightarrow \text{Triphenylformazan.}$$

To Blood Agar Base (Difco) or Tryticase Soy Agar (BBL) 0.005% TTC was added while dissolving, and the medium was then processed according to manufacturer's directions. Plates with and without TTC were swab-inoculated from 24 hour Brain Heart Infusion or Thioglycollate broth. The plates were allowed to dry and sensitivity discs added. The strains tested included: Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus, Streptococcus pyogenes, Proteus vulgaris, and Klebsiella, sp. These strains were isolated from patients at St. Luke's Hospital.

In all cases red formazan developed in the growth of bacteria, well within 24 hours of incubation. Most cultures developed the color in 4 to 6 hours. The inhibition zones were clear while the growth on the remainder of the plate showed varying degrees of redness. The effect of the formazan was especially helpful in reading the inhibition zones on the plates inoculated with Streptococci. The zones on the control plates without dye were often difficult to see while those on the TTC plates were easily detected. Resistant colonies within the zones could be easily seen by their red color.

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A SIMPLE SCREENING TEST FOR ABNORMAL HEMOGLOBINS AND THEIR TRAITS*

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This paper is not being presented because its subject matter is highly intricate, complex and scientifically astonishing; but rather because of its utter simplicity and usefulness to hospitals and laboratories serving patients, particularly those of the Negro race and individuals native to the countries surrounding the Mediterranean Sea.

It is common knowledge that many useful discoveries have been made by empiric observation. In using a special cyanmethemoglobin reagent** this laboratory was among the first to confirm that some of the blood samples did not hemolyze completely. When examined against a light background the tube appeared cloudy or opalescent rather than clear. At first, this was thought to be a defect in the reagent, but additional investigation led us to believe it an asset instead, for it appeared we might have at our disposal an acceptable screening method to suggest the presence of abnormal hemoglobins and their traits. These abnormal bloods could be detected in the course of doing routine hemoglobin determinations.

Materials and Methods

Failure of red blood cells to hemolyze completely when 0.02 ml. of blood is added to 5.0 ml. of this cyanmethemoglobin reagent depends on its carefully controlled hypotonicity and upon the presence of erythrocytes which are abnormally resistant to hypotonic solutions. HYCEL, Inc., has furnished the following formula*** for a reagent which may be made and used in laboratories for medical or research purposes:

Sodium Bicarbonate	10.0 gm.
Potassium Ferricyanide	2.0 gm.
Potassium Cyanide	1.5 gm.
Sodium Chloride	10.0 gm.

Grind to 200 mesh or the equivalent. Dissolve. 2.75 gm. in 1000 ml. of distilled water. Stability does not exceed one week.

In all instances investigated by us, the presence of these abnormally resistant cells was associated with a rather severe microcytic anemia, and in all but exceptional cases with the presence of abnormal hemoglobins. These were verified by paper strip electrophoresis. In microcytic anemias, there are varying numbers of red blood cells which have a large surface area in proportion to the cell size, even though they are microcytes. These are the abnormally resistant cells. It has been observed in this laboratory that there are other clinical conditions besides abnormal hemoglobin disease and trait which contain abnormally resistant cells and consequently fail to hemolyze completely in the reagent. These are severe dietary (milk) anemia; severe jaundice with hepatitis; reversed albumin-globulin ratio; cachexia with extensive carcinoma.

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** HYCEL Cyanmethemoglobin Reagent
*** Based upon patent applications of HYCEL, Inc. Formula may be used for scientific or investigational purposes. Manufacture for sale or distribution expressly forbidden except by license from HYCEL, Inc.

Technic

The technic employed is very simple. It consists in merely expelling 0.02 ml. of blood into a tube containing 5.0 ml. of the cyanmethemoglobin reagent. The tube is inverted several times to ensure thorough mixing. It is then examined against a white background. If no cloudiness is observed, the specimen is read in the photometer and the hemoglobin results are reported as usual. If cloudiness is observed after thorough mixing, 5.0 ml. of distilled water is added to clear the solution, thus enabling it to be read in the photometer. The results are multiplied by two and the hemoglobin value thus obtained can be reported. For further investigation of the specimen showing cloudiness, a sickle cell preparation, using sodium-meta-bisulfite, is made and an electrophoretic hemoglobin pattern is run.

The results obtained by investigating bloods which show incomplete hemolysis in this special cyanmethemoglobin reagent have proved to be of great interest to us, and therefore we present some of the data gleaned over a year's period in the hope that it may be of value to others.

Abnormal Hemoglobin Traits: Importance of Discovery

Everyone is well aware of the fact that patients having abnormal hemoglobin in the homozygous state or possessing two abnormal hemoglobins in the heterozygous state can pose a disturbing clinical problem; however, little has been said of sickle cell or hemoglobin C trait. The physicians on our staff have come to regard this condition more seriously, particularly when a patient is subjected to the stress of a lowered oxygen level. To clarify the picture, a brief rundown of the laboratory findings on one or two patients from each of the services is presented.

Pediatrics

This screening test has been of inestimable value to our physicians on the pediatric staff. Some of the children sent to the hospital with admitting diagnoses of penumonia, upper respiratory infection, possible appendicitis, idiopathic hematuria, or even rheumatic fever have been found to be suffering from acute sickle cell disease, sickle plus C disease, sickle cell trait or hemoglobin C trait.

Case I—Figure 1:

One child admitted to the hospital for a simple tonsillectomy had incomplete hemolysis of the red cells in the cyanmethemoglobin reagent. A sickle cell preparation using sodium-meta-bisulfite was made and found to be positive. Since the child was also anemic, surgery was cancelled and she was treated medically until it was felt that she could tolerate the operation. Electrophoretic studies showed her to have acute sickle cell disease. She had approximately 95% S. hemoglobin and about 5% fetal hemoglobin.

Case II—Figure 1:

Another child was admitted to the hospital from an outlying town with a diagnosis of rheumatic fever. He had joint pain associated with a history of sore throat and fever. When the routine hematology was done it was noticed that the hemoglobin solution remained cloudy, so a sickle cell preparation was made. It was positive. An electrophoretic

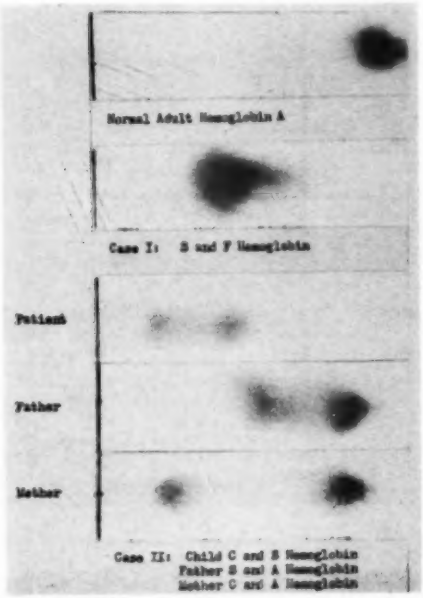

Figure 1. A Simple Screening Test for Abnormal Hemoglobins and Their Traits

hemoglobin pattern revealed that the child had sickle plus C disease. Since the ASO titer was high it is felt that the presumptive diagnosis was correct but that the hemoglobin disorder most certainly contributed to the severity of the symptoms as well as the enlargement of the child's spleen.

Case III—Figure 2:

Still another patient, eighteen months of age, came to the hospital for observation following a bump on the head. Here again, the hemoglobin solution remained cloudy after thorough mixing. The sickle cell preparation was negative but an electrophoretic pattern showed the child to have hemoglobin C trait. The interesting feature of this case was not so much the child, as the father. This man had undergone a subtotal gastrectomy some years previously. His attending physician has been battling a chronic anemia in him ever since, attributing it to a surgically induced pernicious anemia. An electrophoretic pattern was done, revealing that he too possessed hemoglobin C trait (approximately 50%). This might indicate that hemoglobin C rather than the surgery is responsible for his persistent anemia.

Internal Medicine

Case IV:

We helped to verify a diagnosis of Thalassemia Minor for another

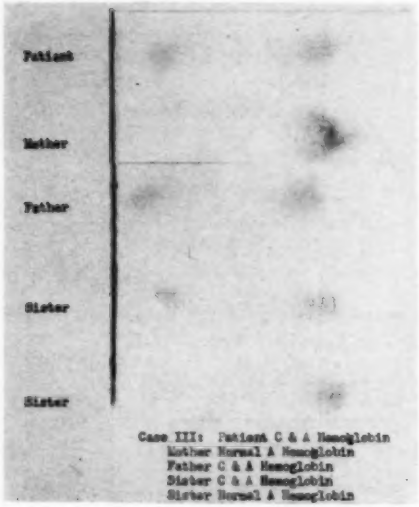

Figure 2. A Simple Screening Test for Abnormal Hemoglobins and Their Traits

hospital. The patient, a woman fifty-one years old, had a cloudy hemoglobin solution and blood for electrophoretic study was sent to us. The hemoglobin was 100% adult; however, red cell indices coupled with the fact that the patient was born in Sicily indicated the clinical diagnosis of Thalassemia Minor.

Obstetrics and Gynecology

While it is general knowledge that women with sickle cell or sickle cell C disease have a difficult time during pregnancy, not too much is known or recorded about those who merely possess the trait. It is true that many of them experience no difficulty whatever, but some of the obstetritians at our hospital feel that if deep anesthesia is a possibility, as for Caesarian Section, complications are apt to arise. The chief problem seems to be an oozing type of bleeding which occurs for no apparent reason. This can be severe enough to cause a significant drop in hemoglobin. Blood transfusions are avoided whenever possible since sickle trait patients do not respond as usual to transfusions; that is, the amount of blood necessary to raise the hemoglobin to a satisfactory level is far greater than the amount needed for a person with normal hemoglobin. It is the opinion of many of the doctors that the reduced oxygen tension of the blood, which might occur during anesthesia, causes the red blood cells to sickle in a manner similar to that of sickling in a laboratory preparation. As a result of the sickling, symptoms of the acute disease may be produced to a variable degree.

Case V—Figure 3:

A patient fitting this general description was admitted to the hospital for a supposedly uncomplicated delivery. Since we were not using the cyanmethemoglobin reagent at the time, it was not known that this patient possessed the sickle cell trait. Capillary oozing was noticed when the surgeon was closing the abdomen. However, as it appeared slight the patient was returned to her room. A repeat hemoglobin was done the next morning and was much lower than the one done at the time of admission. Subsequent studies proved the presence of sickle cell trait. Had the doctors been aware of this insidious disease perhaps a spinal or other kind of anesthetic would have been given.

At a later date the patient's own mother (Figure 3) was admitted to the hosiptal for a gall bladder ailment. Surgery was deemed necessary, so the patient's nephew presented himself as a blood donor. We were now using the new cyanmethemoglobin reagent and as a result both were found to have sickle cell trait. The anesthetist, being forewarned, maintained an oxygen level during the operation sufficient to avoid any complication. The blood bank rejected the nephew as a donor.

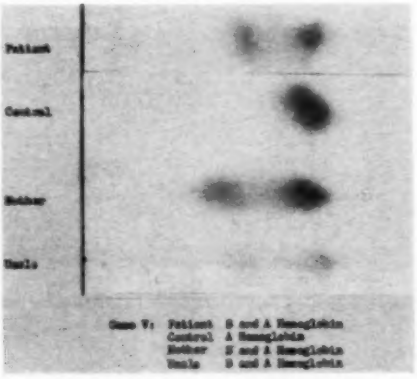

Figure 3. A Simple Screening Test for Abnormal Hemoglobins and Their Traits

On two occasions cloudy hemoglobin solutions were noted on patients suffering from an undiagnosed uterine bleeding. One was a known sickle cell patient discovered on a previous admission; the other proved to have a C plus A trait when an electrophoretic strip was done. The tissue reports on the two patients were hyperplastic endometrium and endometrium with secretory activity. It would seem that the abnormal hemoglobin present in each of these patients could have been a contributing cause of the uterine bleeding.

Surgery

Surgical patients present a similar problem, an oozing type of bleeding when subjected to deep anesthesia, as well as a poor response to transfusion therapy. It is easy to see that previous knowledge of sickle cell trait is a decided advantage when extensive surgery is planned.

Case VI—Figure 4:

A patient of this type happened to be the sister of one of our physicians. She was to undergo surgery for the removal of degenerating fibroid tumors. Her routine laboratory work was satisfactory, but since we were not then using the special cyanmethemoglobin reagent, it was not discovered that she had sickle cell trait. She went through the operation with no apparent difficulty but when the hemoglobin was checked the next morning it had dropped considerably. A pint of blood was given in an attempt to restore the hemoglobin to its pre-operative level. Five hundred ml. of blood fell far short of doing this and since there was no evidence of internal bleeding a sickle cell preparation was made. It was positive. The patient's post-operative course was a trifle stormy but she did respond to medication and was discharged without further difficulties. There is little doubt, however, that if the anesthetist or surgeons had been aware of this added danger to her well-being, more caution would have been used with regard to maintaining a sufficient oxygen tension during the operation. This patient's cells failed to hemolyze completely, and the electrophoretic strip illustrated was obtained at a later date.

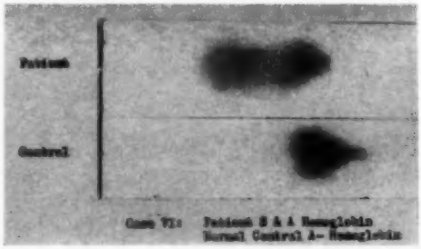

Figure 4. A Simple Screening Test for Abnormal Hemoglobins and Their Traits

Urology

One of the most unusual situations that has come to our attention is a previously unexplained hematuria in people who have proved to be suffering from sickle cell or hemoglobin C trait. Oddly enough, this hematuria seldom lowers the hemoglobin level to any significant degree. I.V. pyelograms and cystoscopy studies show no organic pathology to account for the bleeding, but sickle cell trait or hemoglobin C trait has been present in all. The special cyanmethemoglobin reagent helped us to ferret out many of these. When a relative of one of our hospital employees manifested this disease we were in the fortunate position of being able to discover the sickle cell trait in all the children of her family.

The above case with its hereditary ramifications leads us to believe that perhaps intermarriage between two such families should be discouraged. As a means to this end the described screening test is proposed for prospective marriage partners within the Negro race. One is prompted to deem it of perhaps more value to these people than the Wassermann.

Blood Bank and Transfusion Service

It is here that we would like to discuss the merits of including this screening test as a routine procedure for the selection or rejection of blood donors. We think it not only has value in weeding out unsuitable donors, but serves as an added protection to the transfusion recipient. We are exceedingly aware of the fact that transfusion therapy is aimed at restoring the blood elements of the patient with the least possible embarrassment to his system as a whole. Are we truly doing this when we impart to his circulation varying amounts of abnormal hemoglobin or cells that will sickle if the oxygen content of the blood drops sufficiently? More than likely we are not, since the sickled cells have an increased mechanical fragility and are in a position to do more harm than good.

Again, there is the matter of storage. Is the cell-life span of the sickle cell donor as long as that of the normal donor, and will not the actual drawing of the blood reduce the number of viable cells considerably? Though much of this is conjecture, these possibilities incline us to believe that a sickle trait person is not qualified as a blood donor.

Aviation

In conclusion, I would like to relate a recent occurrence that poses an interesting problem. Not long ago one of the major airlines was obliged to discharge their first Negro hostess. She possessed the sickle trait which caused her to have symptoms of small infarcts of the spleen when she was subjected to high altitudes. It might be well for the various air services, civilian and military, to have a simple screening test of this sort available.

With the jet age here to stay and supersonic flights becoming more and more common, a test of this sort could be a boon to the Air-corps as an aid in the selection of personnel whom they may wish to train as pilots, navigators and the like. Looking further into the future, we are confronted with the many complexities of space medicine. Certainly in this sphere it would be absolutely essential to screen out all personnel who are adversely susceptible to radical changes in atmospheric conditions.

Discussion

We feel that the effort put forth by our laboratory on this project has been well spent as it has stimulated much interest in these disorders and has been the starting point of many interesting discussions. We realize that further development and study are essential. We have already noticed that sickle trait patients do not always have cells present with a surface area large in proportion to the cell size. As a result there are sometimes hemoglobin solutions that are clear in spite of the fact that abnormal hemoglobin is present. We do know that when the oxygen tension of the blood is reduced for any reason, and in the sickle trait patient this reduction can be very little, the phenomenon recurs producing a cloudy solution with this special cyanmethemoglobin reagent.

We present this paper in the hope that other laboratories will try it and thereby give us a realistic picture of the potential merit and value of this test.

Summary

A screening test to aid in detecting the presence of abnormal hemoglobins and their traits is described. The technic is given and the reasons for its adoption are discussed.

Acknowledgment

I would like to express my gratitude to my fellow technologist, Mrs. Mary Greer Johnson, M. T. (ASCP) for her invaluable assistance. I would also like to thank the members of the Staff of St. Elizabeth's Hospital and Dr. David Braden for permitting us to present their case histories.

REFERENCE

A Laboratory Manual on Abnormal Hemoglobins, J. H. P. Jonxis, T. H. Huisman. Blackwell Scientific Publications, Oxford, 1958.

ABSTRACT

REED-STERNBERG CELLS IN THE PERIPHERAL BLOOD AND BONE MARROW IN HODGKIN'S DISEASE

One form of Hodgkin's disease is the so-called abdominal form, in which one or several of the deep-lying abdominal lymph-node groups are the only ones enlarged to any extent. Two cases of this type are reported in this article. Reed-Sternberg cells are defined as huge lobulated and segmented cells with irregular scattered chromatin granules and 1-2 oxyphilic nucleoli, seen in the lymph nodes of Hodgkin's disease.

Case 1: Laboratory findings: (ranges from counts of 5 different days) Hgb. 8.7-10.7 gms. %; RBC 2.5-3.0 M; Reticulocytes 0.8-2%; Normoblasts 0-2/100 WBC; Hct 29 vol. %; MCV 117.4 cu. μ; MCHC 29%; MCH 33.5 $\mu\mu$gm; WBC 2,400-7,800; Giant histiocytes and Reed-Sternberg cells 1-3% of WBC. Photographs of Reed-Sternberg cells are shown, their size ranging from 25-55μ in diameter.

Case 2: Laboratory findings: macrocytic anemia, with many RBC containing Howell-Jolly bodies and/are Pappenheimer bodies. Siderocytes 3 plus. Heinz bodies were also seen. Nucleated RBC, mainly with pyknotic nuclei were numerous and reticulocytes were increased. Leukopenia (neutropenia) and thrombocytopenia were present. Hgb. 4.5-5.6 gms. %; RBC 1.0-1.3 M; Reticulocytes 9.3-11%; Nucleated RBC 2,500-5,700/cu. mm.; Neutrophils 350-780, Monocytes 260-884, Lymphocytes 816-2, 210/cu. mm.; Platelets 6,000-9,000/cu. mm. All figures are ranges of 6 blood counts. Hct. 20 vol. %; MCV 150 cu. μ; MCHC 31.5%; MCD greater than 9μ. One photograph of peripheral blood is presented. Varadi, S., City General Hospital and Wharncliffe Hospital, Sheffield, England. *British Medical Journal* April 23, 1960, pp. 1239-1243.

A COMPARATIVE STUDY OF THE HISTOLOGIC TECHNIQUES FOR DEMONSTRATING CHROMAFFIN CELLS*

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Introduction

Since it is recognized as important to demonstrate the minute granules in the cytoplasm of the neoplastic cells in the diagnosis of pheochromocytomas, it is equally important that the technologist in the pathologic anatomy laboratory be cognizant of the various technics used in their demonstration. In addition to this, he must be aware of the importance of prompt and accurate methods of fixation.

Methods

In 1946, Gomori[1] reported on the use of Heidenhain's Modification of Mallory's Aniline Blue Collagen Stain for the demonstration of the chromaffin cells of the adrenal medulla. He recommended that the tissues be fixed in aqueous formalin, Bouin's fluid or Heidenhain's mercuric chloride formalin. In Gomori's modification, he reduced the azocarmine G from 0.1 to 0.05 g. and altered the staining time to 60-90 minutes at 55-60° C. The aniline alcohol differentiation is controlled microscopically until the chromaffin cells in the medulla are deep pink and form a sharp contrast to the pale cells of the cortex. The tissues are stained in Mallory's aniline blue-orange G or aniline blue-quinoline yellow mixture for 15-40 minutes after mordanting in a 3 per cent aqueous solution of phosphotungstic acid. When quinoline yellow is used in place of orange G, the cortex cells are greenish yellow instead of dull orange and they produce a more striking contrast with the purplish red granules of the chromaffin cells.

In 1939, Bröster[2] reported on the demonstration of adrenal cells with ponceau-fuchsin. The tissues are preferably fixed in formalin-saline. The paraffin sections are deparaffinized and routinely hydrated to water before placing them in a potassium dichromate-sodium sulfate mordant at room temperature overnight. After thorough washing, the sections are stained in ponceau-fuchsin for five minutes, washed and differentiated in a 1 per cent aqueous solution of phosphomolybdic acid and stained with a saturated aqueous solution of aniline blue. Since the chromaffin cells are fuchsinophilous, they are stained red and the collagen is blue.

The Schmorl[3] ferric ferricyanide reduction test prescribed a 5 minute interval in a freshly prepared mixture of equal volumes of 1 per cent ferric chloride and freshly prepared 1 per cent potassium ferricyanide. In 1951, Lillie[4] did considerable experimentation with this technic and used a solution containing 4 ml. of a freshly prepared 1 per cent potassium ferricyanide solution, 30 ml. of 1 per cent ferric chloride solution and 6 ml. of distilled water for 10 minutes at 20-30° C. The sections are washed in 1 per cent aqueous acetic acid and counterstained in a 1:5,000 dilution of new fuchsin in 1 per cent acetic acid. Primary chromate fixation is critical with this technique.

* Second Award, Scientific Products Foundation in Histology, 1960. Read before the 28th Annual Convention of ASMT, Atlantic City, New Jersey, June 1960. Received for publication January 1960.

With the periodic acid leucofuchsin reaction,[5] after fixation in Orth's fluid, the tissues are oxidized in 0.5 per cent aqueous periodic acid, washed in distilled water and immersed in Schiff reagent for 15 minutes. Three sulfite rinses of two minutes each remove the excess leucofuchsin from the sections. Following thorough washing in running tap water, the sections are routinely dehydrated and mounted. With this technique, the chromaffin cells are portrayed in dark red.

A simple technique used in our laboratory, depends entirely on primary chromate fixation in Orth's fluid. The tissues, after fixation, are washed for at least six hours under running tap water, routinely processed in the Technicon and sections are cut at six microns. The sections are deparaffinized, hydrated to running tap water for five minutes and stained for 10 minutes in Mallory's[6] aqueous alum hematoxylin. After staining, the sections are washed under running tap water for 10 minutes, routinely dehydrated through alcohols, cleared and mounted in Permount. With this technique, the nuclei are blue and the cytoplasmic granules in the chromaffin cells yellow to brown. The technique is entirely contingent on prompt and adequate fixation in a primary chromate fixative.

The Gomori-Burtner[7] Methenamine Silver Method for Argentaffin Cells has been applied to the demonstration of chromaffin cells in our laboratory. The deparaffinized sections are treated with Weigert's iodine solution for one hour at room temperature, bleached with 5 per cent sodium thiosulfate and thoroughly washed under running tap water. After several rinses in distilled water, the sections are impregnated with a solution containing 30 ml. stock methenamine silver solution and 8 ml. Holmes' pH 7.8 borate buffer for 3-3½ hours at 60° C. The tissues are rinsed in distilled water, toned in 0.1 per cent gold chloride, rinsed in distilled water and fixed in a 5 per cent aqueous solution of sodium thiosulfate. After thorough washing, they are counterstained in 0.1 per cent safranin 0 in 0.1 per cent aqueous acetic acid or 0.1 per cent light green SF in 1 per cent aqueous acetic acid. The granules in the cytoplasm of the chromaffin cells are black in this technique.

Results

With the Gomori[1] aniline blue-quinoline yellow technique, the nuclei are red and the cytoplasm in the medullary cells lavender to purple. A comparative picture of the cortex shows similarly shaped nuclei and the bright blue staining of connective tissue elements. With this technique, however, alpha cells of pancreatic islets, some cells of the anterior pituitary, neutrophilic leukocytes and myelocytes also possess granules which stain violet to deep purplish red. It would seem, therefore, that this technique demonstrates other cell granules of the chromaffin cells and not chromaffin itself.

The ponceau-fuchsin adrenal stain of Bröster[2] as cited in the Armed Forces Institute[8] Manual of Histologic and Special Staining Technics, demonstrates the fuchsinophilic chromaffin cells in red and connective tissue elements in blue. This technique does not require primary chromate fixation but uses a dichromate mordant after fixation in formalin-saline.

The histochemical reaction of Schmorl[3] demonstrates the sites of reducing substances in the chromaffin cells in blue green against pink to purple nuclei. In our experience, fixation in a primary chromate fixative such as Orth's or Möller's fluid is critical, since the reaction is not demonstrated in tissues of identical cases fixed in either Bouin's fluid or formalin.

According to Lillie,[9] the theory of the positive reaction of chromaffin cells with the periodic acid Schiff histochemical technique has not been fully explained chemically in relation to the Raper adrenochrome hypothesis and requires further study. In demonstrating the chromaffin cells with this technique, we have found it advantageous to counterstain with a 0.1 per cent light green SF in 1 per cent aqueous acetic acid or with Harris' hematoxylin. With the PAS-hematoxylin, the nuclei are blue against the red purple cytoplasmic granules in the chormaffin cells, whereas the application of light green as a counterstain produces green background staining against similar red purple cytoplasmic granules in these cells. We have not successfully demonstrated these cells in medullary tissue of the adrenal except in those cases where primary chromate fixation was used.

The staining of adrenal medullary tissue with Mallory's[6] aqueous alum hematoxylin after fixation in Orth's fluid produces yellow to brown cytoplasmic granules in the chromaffin cells against clearly demonstrated blue nuclei.

Chromaffin cells have the same property of reducing diamine silver salts as that found in enterochromaffin or argentaffin cells. Masson[10] considered the argentaffin cells as part of the chromaffin system. The technic of Gomori-Burtner[7] for argentaffin cells has been used in this section of a pheochromocytoma to demonstrate the cytoplasmic granules in the chromaffin cells. Primary chromate fixation with Orth's fluid was used in this case and alternate slides were counterstained with Safranin O and light green SF.

Discussion

The parenchymal cells in the adrenal medulla have large vesicular nuclei, an irregular columnar shape and usually an aggregation of secretion granules at the venous pole. The cytoplasm of the cells is filled with minute granules that react with the chromium salts, or other metals, to yield a yellowish or brownish color. The cells are, for this reason, termed chromaffin cells or chromaffin tissue. These granules are believed to result from an oxidation of the hormone of the adrenal medulla, epinephrine. The chromaffin cells are unique in that they combine some of the characteristics of the nerve cells with those of secretory cells.

Potassium dichromate is ordinarily used to demonstrate the chromaffin reaction and most of the techniques here given depend on prompt and adequate fixation in a primary chromate fixative such as Orth's fluid. Usually chromate fixation is prescribed, but, to a lesser degree, chromaffin substances can be rendered demonstrable after fixation in neutral formalin and mordanting in potassium dichromate. The presence of acid in either the formalin or bichromate fixative may prevent chromaffin from staining in the same material as that which will give good staining

after fixation in Orth's fluid. It is advisable, when possible, on those tissues on which a chromaffin reaction must be demonstrated, in particular a pheochromocytoma, to fix pieces of the tumor in three fixatives; Orth's fluid, neutral buffered formalin and Bouin's fluid.

Summary

It can be seen that there are a wide variety of methods available for the demonstration of the chromaffin reaction, all of which can be reproduced under carefully controlled conditions. It would seem, however, that the simple technique of primary chromate fixation followed by staining in Mallory's[6] aqueous alum hematoxylin is as good as the more involved and cumbersome procedures.

Acknowledgments

I would like to express my sincere appreciation to Dr. H. T. Enterline, Associate Professor in Surgical Pathology and Pathology and to Dr. John J. Moran, Associate in Surgical Pathology and Pathology, University of Pennsylvania, for their contributions to this paper in the evaluation of the techniques for demonstrating chromaffin cells in pheochromocytomas.

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THE INTRACUTANEOUS INOCULATION OF GUINEA PIGS AS A MEANS OF DETERMINING THE VIRULENCE OF ACID-FAST BACILLI, WITH SPECIAL EMPHASIS ON THE ANONYMOUS (ATYPICAL) ACID-FAST BACILLI AND ISONIAZID-RESISTANT TUBERCLE BACILLI. I. PRELIMINARY REPORT[1,2,*]

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INTRODUCTION

The value of the guinea pig test for virulence determination of acid-fast bacilli has markedly decreased, as so many of the isoniazid-resistant and so-called anonymous acid-fast bacilli show little or no virulence for guinea pigs.[1,5,10,11,13,16,17] Subcutaneous, intraperitoneal and on occasion even intracardial inoculation of large doses of these bacterial suspensions into guinea pigs elicits very little macroscopic evidence of tuberculous involvement. There exists, therefore, a need for a reliable virulence test for the troublesome groups of isoniazid-resistant and anonymous acid-fast bacilli.

It was thought that perhaps the intracutaneous inoculation of guinea pigs, a technique which proved useful in distinguishing saprophytic from virulent mycobacteria,[6,9] also might provide a rapid, more reliable means of determining the virulence of these questionable cultures. Thus, the present study was set up in the hope of evaluating the following: 1) the reliability of the intracutaneous test as an indication of the virulence of acid-fast bacilli for man; 2) comparison of the intracutaneous, subcutaneous and intracardial routes of inoculation as measures of the virulence of acid-fast bacilli.

MATERIALS AND METHODS

Cultures used: The studies herein reported deal only with stock cultures of acid-fast bacilli. These include the following: five representatives each of human and bovine strains; seven avian strains, four of which were obtained from Dr. D. W. Smith, University of Wisconsin, Madison; six saprophytic stock strains; three nocardia; seven isoniazid-resistant human strains, four of which were obtained from Dr. A. A. Grover, Christ Hospital, Cincinnati, Ohio; thirty-six representatives of the four groups of atypical acid-fast bacilli, supplied through the courtesy of Dr. E. H. Runyon, Veterans Administration Hospital, Salt Lake City, Utah; nine cultures of *M. fortuitum*, eight from the collection of Dr. D. W. Smith and one from Dr. E. H. Runyon.

Preparation and inoculation of cultures: Using the Hopkins tube technique[8] for standardization, cell suspensions were prepared from fresh egg slant subcultures and diluted serially in physiological saline solution. For intracutaneous inoculation, the final wet weight of organisms inoculated were 0.1 mg., 0.01 mg. and 0.001 mg., the inoculum in each instance being suspended in 0.1 ml. of saline. Two sets of duplicate inocula were injected into the shaved abdominal surface of one guinea

[1] From the Tuberculosis Unit, Communicable Disease Center, P. O. Box 185, Chamblee, Georgia.
[2] Presented in part before the Medical Session, as part of 2B, at the annual meeting of the American Trudeau Society, Chicago, Illinois, May 25, 1959.
[*] Received for publication April, 1960.

pig, one inoculum into each of the quadrants adjacent either to the axillary or inguinal regions. Animals were examined twice weekly and changes noted at the site of inoculation were recorded. For purposes of comparison, other guinea pigs also were inoculated subcutaneously and intracardially using inocula containing 0.1 mg. and/or 1.0 mg. wet weight of bacilli.

RESULTS

The pathologic changes observed following intracutaneous inoculation ran the gamut from no visible lesion to a persistent, open ulcer. This latter served as a criterion for a positive intracutaneous test; microscopic examination of the exudates from these lesions shows them to be teeming with acid-fast bacilli. In most instances positive intracutaneous tests could be recorded in 8-14 days, and only rarely did it take 21 days for the reaction of any of the cultures to manifest itself. In Table I are given the results obtained when stock cultures of acid-fast bacilli were submitted to this test. For comparative purposes, the results of subcutaneous and intracardial inoculation also are recorded. The severity of gross tuberculous infection following subcutaneous and intracardial challenge is given an arbitrary value from ± to 5+ after the scheme used in this laboratory.[8] It can be seen that human, bovine and avian strains produced open ulcerations at the site of inoculation, whereas all but one of the saprophytes were negative, or at most had a small area of induration which faded in a few days. One saprophyte in our laboratory culture collection consistently produced an open

TABLE I—Results of Intracutaneous Inoculation of Guinea Pigs with Stock Strains of Acid-Fast Bacilli. Comparison with Subcutaneous and Intracardial Routes of Challenge

Cultures	Intracutaneous (mg)*			Intracardial**	Subcutaneous**
	0.1	0.01	0.001		
Human (5 strains)	OS	OS	OS	4—5+	4—5+
Bovine (5 strains)	OS	OS	OS	4—5+	4—5+
Avian (7 strains)	OS	OS	OS	—	—
Saprophytes (5 of 6 strains)	— or Indur.	—	—	—	—
Nocardia (2 of 3 strains)	OS	OS	not done	—	—
Photochromes (I) (11 of 12 strains)	OS	OS	OS	±	—
Scotochromes (II) (2 of 4 strains)	OS	Indur.	—	—	—
Battey (III) (7 of 11 strains)	OS	OS or Indur.	—	±	—
Rapid Growers (IV) (2 of 9 strains)	OS	OS	—	—	—
M. fortuitum (2 of 9 strains)	OS	OS	—	—	—
IN H-Resistant Human (6 of 7 strains)	OS	OS	—	— to 2+	—

* OS = Open sinus.
Indur = induration.
— = no visible change.

** Gradations from + to 5+ indicate severity of gross tuberculous infection following inoculation. Necropsies performed 6 weeks post-infection.

sinus on intracutaneous inoculation. Further studies indicated that this organism actually might be a member of one of the groups of atypicals; with this latter thought in mind, subcultures of the organism have been sent to several other laboratories for further study. Six of the seven isoniazid-resistant human strains, although producing little or no disease when inoculated subcutaneously, yielded open lesions on intracutaneous inoculation (See Fig. 1). Two of the three species of *Nocardia*

FIG. 1. **Results following intracutaneous inoculation of four INH-resistant human strains; all four strains were innocuous when administered subcutaneously. One strain produced no lesion at the inoculation site (see black circle) while the other three showed varying degrees of reactivity.**

(*madurae* and *asteroides*) produced persistent open ulcers whereas *N. braziliensis*, usually regarded as less virulent than the other two (Dr. Lucille Georg, personal communication), caused no visible pathologic change. The photochromes and Battey strains, generally considered the most virulent of the anonymous acid-fast bacilli, were most consistent in producing ulceration at the site of intracutaneous inoculation, whereas the scotochromes and rapid growers, considered the least virulent of the anonymous strains, were much more variable in the reactions they produced following intracutaneous inoculation (See Fig. 2). These results are somewhat at variance with the data abstracted from the National Tuberculosis Association meetings[7] held in Chicago, May 24-29, 1959. In the abstract it states that ". . . all the anonymous strains, except the scotochromes, produce open, draining abscesses." As will be noted from the data in Table 1, this statement is no longer correct. Of the atypicals tested, two of the four scotochromes and two each of the nine representatives of rapid growers and *M. fortuitum* produce open sinuses on

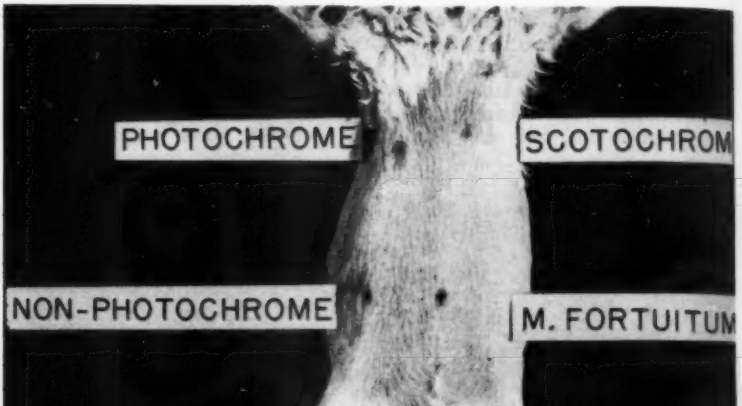

FIG. 2. **Results of intracutaneous inoculation of *M. fortuitum* and representatives of Groups I-III of the Anonymous Acid-Fast Bacilli. Although all four sites have an open sinus, this is not necessarily indicative of the usual reactions observed within each group (see comment in general context).**

intracutaneous inoculation. The reason for the discrepancy in data reported in the abstract is that only a few representatives of each group were studied at the time the abstract was submitted and they reacted as reported. However, more extensive studies revealed the extremely variable results obtained with scotochromes and rapid growers. Of "wild strains" that have been studied to date, roughly 50% of scotochromes and 30-40% of rapid growers produce an open sinus. With these latter two groups, correlation of intracutaneous tests with culture virulence for man is not nearly as good as in the case of photochrome and Battey strains. These results with the atypicals are in accord with data presented by Wayne et al.[14, 15]

If the guinea pigs inoculated by the intracutaneous route were kept for six weeks and then autopsied, it was noted that only human and bovine strains of tubercle bacilli produced progressive tuberculosis in the animals. The other acid-fast bacilli studied, although producing ulceration at the site of intracutaneous inoculation, apparently lacked the power to invade and produce progressive infection. This latter was substantiated by virtue of the fact that these strains appeared essentially avirulent when tested by the conventional subcutaneous or intracardial routes of inoculation.

Although this intracutaneous technique has been favorably tested on more than one-hundred reference cultures submitted to this laboratory for identification, results are being withheld at this time because of the

paucity of correlative clinical information submitted with these cultures. An attempt is being made to gather information relative to source and frequency of isolation of these reference cultures with the hope of including the results in a subsequent report.

DISCUSSION

In recent years strains of anonymous and isoniazid-resistant acid-fast bacilli have been isolated with increasing frequency from patients suspected of having tuberculosis. The fact that these organisms do not produce progressive disease in the guinea pig is no evidence that they are not virulent for man. Frequently these bacilli are isolated repeatedly from sputa and resected tissue in the absence of any other organisms, thereby strongly implicating these atypical acid-fast bacilli as causative agents of disease. The fact that it has been impossible consistently and repeatedly to fulfill Koch's Postulates as regards the anonymous and isoniazid-resistant acid-fast bacilli is no reason why we should negate their importance in human disease, for another widely recognized and accepted human pathogen, the leprosy bacillus, has been very recalcitrant in this same regard. Runyon has stated[12] that there is still an "empty chair" awaiting occupancy by some laboratory animal which is susceptible to infection by these atypical microorganisms. Until this "chair" can be more adequately filled, all outward appearances seem to suggest that the disposition of the lesion formed following the intracutaneous inoculation of guinea pigs appears to provide a much better correlation with culture virulence for man than does the usual subcutaneous, intraperitoneal or intracardial route. As was stated earlier, because of lack of sufficient correlative clinical data, results of practical evaluation of the intracutaneous test are being withheld from this report.

It has been pointed out[6] that in performing the intracutaneous inoculation, it is possible for some of the inoculum to be injected subcutaneously with the ultimate result that no ulcer may be produced by suspected virulent organisms. For this reason we recommend that duplicate inoculums of the same concentration of bacillary suspension be inoculated in diagonally opposite quadrants of the shaved ventral surface of the guinea pig. However, if one is careful to raise a good bleb at the site of intracutaneous inoculation with each and every inoculum, it is possible to inoculate four cultures into one animal thereby providing for considerable economy in this respect.

In our experience we have yet to notice any of the exudate from an ulcer drain into the cage bedding; rather, a coagulum of pus collects at the opening of the sinus and it is necessary to squeeze this lesion in order to get the pus to exude therefrom. It would seem, therefore, that this route of inoculation offers little more hazard to animal caretakers than the subcutaneous route, the site of which also may open and drain.

From the standpoints of speed, possibility of inoculating as many as four cultures per animal, and closer correlation with culture virulence for man, it would seem that the intracutaneous route of inoculation of guinea pigs provides a more reliable "virulence" test for the isoniazid-resistant and anonymous acid-fast bacilli than do the routine methods currently in use.

SUMMARY

A comparative investigation of the intracutaneous, subcutaneous and intracardial routes of challenge for guinea pigs has revealed that the intracutaneous inoculation provides a rapid, reliable test of the virulence of human, bovine and avian strains of mycobacteria and further that it appears to be a more adequate measure of the apparent virulence of isoniazid-resistant and anonymous (atypical) acid-fast bacilli for man than do the more conventional subcutaneous or intracardial routes.

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MUSINGS OF A BY-GONE DAY*

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What has become of the "Do-it-yourself-technician"—the one who never had Prepared Media, Clinistix, or Diagnex Blue kits? She could produce five liters of culture media from ten pounds of hamburger—the fragrance of the hamburger, salt and water cooking in a kettle was guaranteed to prompt a visit from every free loading nurse or interne within smelling distance! Four hours of cooking plus cooling and filtering and finally sterilization. Then into the hungry maws of that monster of copper and temperament, the portable autoclave. Shades of Cape Canaveral! Will it fire, light or backfire with its minor explosion and resultant uproar? Will it maintain pressure or hiss like a mad thing and run dry? Is there enough water on the bottom or will the media float and turn over if the water level is one-half inch too high? Only Miss "do-it-yourself" knows that to leave this monster unattended is to ruin her brew. Will the cotton plugs end up on top or explode into the fiery furnace because she released the valve too soon or be sucked into the bottom because she waited thirty seconds too long? And then there are the little specialties like peptone broth, and the addition of those lively typhoid bugs for your Widal reaction; not to mention the mashing of all those potatoes for the Petragnani. The Petragnani is full of mold! Oh well, just rice another ten pounds of potatoes.

Another fascinating facet of the "do-it-yourself" era was "Operation Sheep Blood," which exploded the entire theory of "gentle as a lamb." First, you gained Sam the Ram's attention with a hypo of pheno-barb; then assembled razor, alcohol, sponges, etc., along with one pathologist and eight strong men. Sam supplied the cells for a number of years until in a display of temperament, he raced round and round a well-meaning Mother Superior (who shall be nameless), lashing her firmly to a tree. Sam's banishment was almost immediate—even sheep can be fired! "Operation Sheep Blood" was replaced with a trip to the local abattoir, where, for fifty cents, a flask was filled with blood that otherwise found its way into the sewage system. Locating the lamb butcher in the midst of cows and hogs and conveying, by hand signals, her needs while looking about for a safe place in the event of a stampede was just another of the many accomplishments of the wonder technician.

When winter arrived, so did the pneumonia bug, and so did the increased need for mice. It seemed that when you needed all the mice available, for some reason or another, the colony was depleted. When you picked your victim, the routine went something like this, "Hold still, you silly mouse, this hurts me more than it does you. He bit me! He got away! He's under the centrifuge! Don't let him get into the wards! Put him in the jar! I'll be glad to autopsy him!"

In those days we made our own typing sera and drew "just a little extra" blood from our patients to type and pool for the process. Who ever heard of a "blood bank?" It was "stick the donor's finger; if he's the right type, stick his arm. The citrate's boiling. See if the O. R. can

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take him now. The matching will be ready in ten minutes. The Kahn is almost finished. Take him to the O. R."

Then there's good old Dakins Solution which we made by mixing sodium carbonate and tap water and bubbling through chlorine gas until the mixture titrated to a pH of 13.5. Never mind how much chlorine, the pressure gauge never works. A lab technician develops a feel. Don't bang the special porcelain filter—if that breaks we'll all be dead. Dakins was discovered in World War I and was still being manufactured in our lab after World War II. Wounds, osteo infections, infected incisors, etc. were irrigated day and night with the stuff and once or twice a day bacterial counts were made. Wondrous cures were wrought but the ulcer rate among M.T.'s soared! I remember the day the chlorine tank cracked and the Fire Department lowered it gingerly down four stories in a rope sling. Such gasping and excitement! We raced for oxygen for our engineer who was trying valiantly to trace the leak and repair it, aided by an ammonia bottle. Chlorine plus ammonia! "Come out friend, you are turning blue!" Of course I am grateful today for this excellent training for A-bomb warfare; I know now when to run and how fast!

It's truly a wonder that so many technicians survived the "rat race" in a room full of typhoid bugs and chlorine gas. But we're a hardy lot and have lived to tell the tale today as we measure out our prepared dry media and call in an order of typing sera which will be delivered tomorrow.

ABSTRACTS

OBSERVATIONS ON 100 CASES OF LEUKAEMIA IN CHILDHOOD

Deaths attributed to leukemia at all ages in England and Wales have increased 2½ times in the last 2 decades. In childhood there is now a peak incidence between the ages of 2 and 4 years.

In 98 patients with acute leukemia, leukocytosis was encountered more often (30 cases) than leukopenia (25 cases). Leukocytosis was nearly always accompanied by abnormal cells in the peripheral blood (26/30); in contrast, cases without leukocytosis frequently had only mature cells in the differential count (32/68). 14 children had a Hgb. over 11 gms % when first seen and in 6 of these leukemic (blast) cells were not seen initially in the peripheral blood.

2 cases were of chronic myeloid leukemia.

The total white cell count was normal in nearly ½ of the 100 cases. Lightwood, R, Barrie, H., and Butler, N., The Hospital for Sick Children, Great Ormond St., London, England. *British Medical Journal,* March 12, 1960, pp. 747-752.

MULTIPLE INHERITED ERYTHROCYTE ABNORMALITIES IN AN AMERICAN NEGRO FAMILY: HEREDITARY SPHEROCYTOSIS, SICKLING AND THALASSEMIA

An American Negro family is described in which there are found the genes responsible for 3 inherited abnormalities of the erythrocyte, namely, the gene resulting in spherocytosis, a thalassemia-like trait and the sickling phenomenon.

Photomicrographs of peripheral blood films of pertinent members of the family are provided. Cohen, F., Zuelzer, W. W., Neel, J. V. and Robinson, A. R., The Child Research Center of Michigan. The Children's Hospital of Michigan, Detroit, and the U. of Michigan, Ann Arbor, Michigan. *Blood,* 14, 7 1959, pp. 816-827.

RETICULUM CELL SARCOMA TERMINATING IN ACUTE LEUKEMIA

Records of 113 patients dying with reticulum cell sarcoma revealed 6 cases whose courses terminated in a syndrome resembling acute leukemia. There was anemia, leukocytosis (20,000-80,000), thrombocytopenia (under 100,000), and a differential count of the blood and marrow revealed a large % of immature cells (35-96%). These were identified as reticulum cells in 3 patients, myeloblasts in 2, and as monocytoid granulocytes in 1.

Photographs of marrow and peripheral blood are presented. Zeffren, J. L., and Ultmann, J. E., Dept. of Medicine, Columbia U. Coll. of Physicians & Surgeons and the Medical Service, Francis Delafield Hospital, New York, N. Y. *Blood,* 15, 2 1960, pp. 277-284.

THE DIRECT METHOD OF ANTIBIOTIC SUSCEPTIBILITY TESTING OF STAPHYLOCOCCI*†

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The results of antibiotic susceptibility tests are usually available 15-18 hours later than the isolation of the organism to be tested. Obviously, placing antibiotic sensitivity discs directly on the inoculum of the primary culture would save one day's delay, if the results are as valid as those obtained using pure cultures. This paper concerns the comparison of such a direct antibiotic susceptibility testing with the common procedure of testing isolated strains in pure culture. The reasons for reporting this are the obvious time advantage if results are comparable, and the need for "simplified office procedures" in the management of cutaneous bacterial infections. Furthermore, changes and improvements in both media and antibiotic sensitivity discs since previous similar studies, and the advent of other antibiotics, required that the procedure be evaluated under present conditions.

Methods

Most cultures were obtained from the skin lesions of out-patients and in-patients seen in the Department of Dermatology of this hospital during the past year, February, 1959 to February, 1960. The diagnoses in this group of patients included impetigo, furunculosis, ecthyma, pustular folliculitis, and secondarily infected dermatoses, particularly, various types of dermatitis. Often, follow-up cultures were taken for epidemiological studies, accounting for some cultures from the nares and normal or healing skin. Specimens were collected on sterile, saline-soaked cotton swabs. A sector, approximately one-third, of a blood agar plate (Trypticase Soy Agar, B. B. L., plus 5% sterile defibrinated sheep blood, Cappel) was inoculated with the swab and streaks were made from this area to obtain isolated colonies. Antimicrobial discs (Sensi-Discs, B. B. L.) of chloramphenicol, erythromycin, and tetracycline (usually 5, 2, and 5 μg., respectively) were then evenely spaced on the innoculated sector. Following sufficient aerobic incubation at 37° C. for growth adequate for recognition of colonies and inhibition zones, isolated colonies of *Staphylococcus aureus* only were picked and antibiotic susceptibility tests repeated on pure cultures grown on Trypticase Soy Agar, usually without added blood.

Results

Of 596 cultures in which the direct and the standard (delayed) tests were compared, 569 were from the skin and 27 from the nares. Of these 569 skin cultures, 64 produced no growth, 105 yielded normal flora only, and 14 had *S. aureus* but with insufficient growth for correct interpretation, 2 yielded *Candida albicans*, and 11 had beta hemolytic streptococci only. The comparison of the two methods of susceptibility testing of staphylococci from skin is based, then, on the remaining 372 cultures

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which yielded *S. aureus* in adequate numbers, either in pure or mixed culture. Of these 372, 358 (96%) gave the same reaction when tested in pure culture and thus resulted in a time advantage, and 25 (7%) of these with the time advantage also offered the additional advantage of permitting recognition of staphylococcal strains having different antibiograms but similar morphology. The direct method was unsatisfactory in 15 (4%), of which 7 were overgrown with species of *Proteus, Pseudomonas,* or other gram negative rods, and 8 were obscured by normal flora.

Of the 27 nasal cultures, one produced no growth, 11 yielded normal flora only, and 3 had *S. aureus* but with insufficient growth for correct interpretation. Of the remaining 12, all resulted in a time advantage, and one of these aided in recognition of strains with different antibiograms. None of these 27 was overgrown by obscuring organisms.

Discussion

Bondi *et al.*[1] modified the penicillin assay procedure of Vincent and Vincent[6] by placing antibiotic discs on plates inoculated with clinical specimens in order to test susceptibility at the time the primary culture is made. They reported the results of 54 selected clinical specimens, only 8 of which contained staphylococci. The present study was made to determine if such a procedure would be useful in bacteriology of the skin where often a large normal flora is present. The procedure used was similar to that of Bondi and his colleagues, except that (1) commerically available discs of chloramphenicol, erythromycin, and tetracycline were used instead of freshly prepared streptomycin and penicillin discs, (2) only aerobic cultures were done, but (3) antibiograms were repeated on the isolates to check the validity of the first interpretation.

The three antibiotics used were chosen because staphylococci are more often resistant to them than to other clinically useful agents such as neomycin and bacitracin, which seldom fail to inhibit them. Nevertheless, a neomycin disc is useful in primary cultures since it is much less inhibitory for coagulase positive than for coagulase negative staphylococci.[3] This permits isolation of the former within zones of inhibition of the latter. Other antimicrobial agents may be used depending on the information desired and the nature of the specimen. Although inhibition zones of some antibiotics may be altered by the presence or absence of blood in the agar, this factor did not cause different final results in these comparisons.

The quality of commercially available discs has improved recently,[2] and the concentrations of antibiotic discs are such that only one disc is needed to predict resistance or susceptibility, within the limits of the test.[5] For the organisms and procedures concerned here, the tube dilution and disc tests have shown good agreement.[4]

Cultures which produce scant or no growth often result from the use of topical or even systemic antibiotics. With cultures which produce sufficient growth of *S. aureus,* this procedure usually yields more rapid results of antibiotic susceptibility. Beta hemolytic streptococci, also of importance in such infections as impetigo, are not submitted to susceptibility testing because of their generally uniform response to antibiotics. Knowledge of the cultural characteristcs of the organisms involved,

however, is essential to a correct interpretation of the results, since overgrowth by resistant (but less significant) organisms may mask the results.

Summary

An adaptation of the procedure of *Bondi et al.*[1] has been used to test the antibiotic susceptibility of staphylococci in primary cultures from the skin and nares. In 372 skin cultures producing sufficient *S. aureus* for testing, 358 (96%) yielded the same results when the organisms were tested in pure culture. Results in the remaining 15 (4%) were unsatisfactory because of masking by resistant normal flora and spreading gram negative rods. Results with nasal cultures were similar. In a few cases, the procedure had the additional advantage of permitting recognition of staphylococcal strains with different antibiograms but similar morphology.

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THE MEDICAL LABORATORY TECHNOLOGIST AS A PROFESSIONAL MEMBER OF THE PATIENT'S TEAM*

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The answer to the questions of "What Is a Profession?" and to "What Constitutes Professionalism of Organization and Action?" is not an easy one. If it can be adequately defined and discussed, it can be applied to the profession we are discussing. We must first get a background of the sociological whole where the technologist serves, then discuss the three facets that determine a profession and professional standing.

The morés of the American people were developed in the context of their moving, as individuals, into what has been termed a new country, a wild country and a dangerous one. It thus developed that the family was the unit of defense, of economics, of education, of religion, and of medical care. As long as there were few homes all phases of the activities of living, eating, teaching, and worshipping took place in individual homes. Complexities brought about by cities, multiple homes and multiple people only changed the character of such morés—not the basic substance. Thus, the hospital, school, and government buildings are extensions of people's homes to care for needs that formerly were a part of home life. The practice of medicine moved to the doctor's office to make equipment available and to enable the physician to see more people. His assistant, the nurse, the technologist, the technician, the aids and the administrators, all developed in his office or the hospital to more adequately serve people who now used his office or the hospital as an extension of their homes.

So the medical team came into being to provide more adequate and, because of organization, less costly service to the people of a community. The physician is the Captain of this team. The members of the team are integrally important as providing diagnostic and therapeutic services of a high order even though coordinated and interpreted by the physician. Each one thus serves patients personally with a personal service even though there are times when they individually may not see that patient.

It is characteristic of a profession such as Medicine, and therefore of the members of the professional medical team, that each is considered to have rendered a personal service to a patient and is individually responsible for acts undertaken in that service. The concept of the hospital and the doctor's office as an extension of the patient's home puts the picture in its proper light. When a technologist approaches a patient in the hospital, he or she must consider himself in that patient's home and conduct himself accordingly. The simple act of inserting a needle into a vein to draw blood is legal assault on the person or the patient in his home (the room at the hospital or the lab at the office) and must have the patient's consent to be legal. I admit that most times it is implied when the patients present themselves for service, but the professional person, physician or technologist, never forgets he serves patients by consent of those served. In fact, the surgeon always requires

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written consent when his services are likely to include "assault" with a knife, as in an operation.

Let us waive abstract definition as to what constitutes a profession by quoting only one or two from the dictionary: "One engaged in one of the learned professions of Medicine, Law, or Theology," or Professionalism is defined as "professional character, spirit, or method." I also want to quote from "The Second Mile," by Wm. E. Wickenden: "Every calling has its mile of compulsion, its daily round of tasks and duties, its standard of honest craftsmanship, its code of man-to-man relations, which one must cover if he is to survive. Beyond that lies the mile of voluntary effort, where men strive for excellence, give unrequited service to the common good, and seek to invest their work with a wide and enduring significance. It is only in this second mile that a calling may attain to the dignity and the distinction of a Profession."

"In this context, the marks of a profession are: first, the type of activity which carries a high individual responsibility; second, a motive of service associated with limited rewards; third, the motive of self-expression which implies joy and pride in one's work and a self-imposed standard of excellence; and fourth, there is a conscious recognition of social duty to be fulfilled."

In general, there seem to be three factors in the profession of medical technology. First, there is the technologist as an individual person, all that goes into his approach to life, the service he renders, the work he does, the skill he requires, and the integrity of mind that permits him to serve honestly, accurately, and cleanly, for the best interests of those served and perhaps against his own better personal advantage. Second, there is the Technological Society that supports the individual in his professionalism, signifies his acceptance among the group of individuals making up the profession, sets its standards of education and skill and gives thought to the ethics of all relationships. Third, there is the acceptance by the community of the medical laboratory technologist as a professional person in the broadest sense, thus actually giving professional standing. All three must go hand in hand. An individual cannot attain professional standing without his society. A society cannot attain professional standing without having professionally minded individuals to make up its group. The acceptance by the public or the community served is dependent on both the organization and the individual.

Now let us analyze these three factors and see how each fits into the whole professional picture.

A profession, in general, has certain characteristics as far as the individual is concerned. In the first place, there is a general attitude of mind, an independence of thought that expresses itself in an individualism of approach to all problems. Such individuals must analyze the circumstances surrounding the problem, arrive at the conclusion by themselves even though they may reach the same conclusion others have, and then work out their own particular mode of action to meet that problem. In general, there is a willingness to assume responsibilities that others may not desire to assume. By the very nature of the method of thinking, the professional-thinking person does not follow

as much as he leads. It can be foreseen that such a person would become an entire business within himself or herself as an individual service entity, and there is, in general, such acceptance of this role for medical people. Add to this individual an objectivity that is profound enough to subjugate pain in himself at times because of intensity of preoccupation or of concentration, an education that will enhance these attributes, and you have an emerging picture of a professional individual. This education may be entirely academic but will include mental skills of a rather concentrated order. It may include manual skills coordinated with the mental skills. It will also include ethics of relationship of people to people. A professional person will be preoccupied with ethics.

To this picture must be added the general nature of the work with which professionals are involved and, in most instances, it may be summed up by the word "service." They serve through their mental and their manual skills delivered personally to the consumers of these skills. The service may possibly deal with the environment in which the individual lives in an attempt to make that environment more agreeable to live in. It may deal with services to the individuals in their environment to make them more adaptable to that environment. It may deal with some ramification of such services to point out to the individual the most useful way on interpreting and using the enviroment.

This is a broad picture of a very difficult concept. Basically, it is of an individual who is objective enough in his service to those whom he serves to make him oblivious to his own interests. At the same time, he finds himself faced with the problem of personally looking after his own interests in the way of selling his professional knowledge and skill. Suddenly into what might be a reasonably clear picture are injected questions of economics which, if mixed without clear thoughts, become very confusing. The professional person is the negotiator of his business relationship, the negotiator of the amount received in recompense for his skills, and the negotiator for the conditions under which he will serve. The professional individual, therefore, is usually his own business agent. He may depend only on himself or he may voluntarily combine with others to get better results. He must carefully weigh each method of negotiation and act in accordance with the one selected. But, in general, he will depend on himself.

A professional society is as necessary to the professional man or woman as the education and special training which make him valuable. His professional society has very definite responsibilities. It must uphold standards of individual conduct, standards of professional conduct, and develop for the entire group a *pool of experience*. Herein lies a particular responsibility which is a basic difference between a social organization and a professional one.

Professional people attend meetings of their society and bring to all other professional members of the society their day-to-day experiences in the practice of their profession. This is contrary to other businesses which have a tendency to be secretive in their processes, to gain copyrights on their techniques and on their special developments so that they may *personally* gain from it. It is admitted that professional people working for large businesses find themselves curbed by rules as to how much

they can tell, but the principle is just the same. A professional man or woman donates new knowledge to his society for the development of all in the service which they render. When he discovers a new method of approach, he writes about it, and the society prints his writing. When he develops a new technique, he tells about it at his professional meeting, sharing it with all others.

In return, his professional society gives him standing with himself, with his colleagues and with those he serves. Insofar as such an association develops a depth in its pool of experience, will it develop in the minds of the community as a professional society. Insofar as it develops its ability to serve those who are serving others (and thus make them more responsible, more objective, more professional and more ethical), will it develop a professional status in the minds of the community. In so doing, it applies that professional status to the individuals that make up the organization.

The third facet of professionalism is equally difficult to describe or discuss. For this purpose, let us for a moment be the users of services we consider to be professional. We would certainly want to know of the education, training and experience of the person or persons we select to serve us. We would want to feel that we could go to them and get an objective analysis of any problem we have and a recommended solution that would be solely in our interest. We would want to know of his associates, i.e., his society, which we would expect to set his standards, his ethics, and help him to live up to them. We would then look for certain integrities, certain skills, and certain trainings that would serve our needs. It may be that we would not seek out an individual professional person but a group of such persons, and in such an instance we would expect the group to meet those standards of integrity, training, experience, and honesty in service and teamwork that we wanted in an individual. We would expect that the leader of the group to whom we go and who represents the group, would have selected the other members of the group with the same care we would use.

When we, as users of Medical Technologist service can feel as above about ourselves, as givers of that service, we are ready to be considered professional. When we can present the skills, the readiness to assume responsibility, the deep humility that keeps us continually learning, the integrity to reach accurate conclusions, and the exercise of judgment based on experience, we can then ask for acceptance into the professional fold.

Let me take one moment to discuss one facet of professional organization not generally given much consideration. In its purest sense, a professional organization is not involved in the economics of the individual members except where the economics impinge upon ethics. The society may possibly set up certain standard economic agreements for the use of its members, but, as a rule, it is only as binding as the opinions in the society can make it. In such a society, the individual members do their own economic bargaining for fees, wages, hours, vacations, etc. It is perfectly possible, however, for such an organization to decide to operate as a collective bargaining unit and ask that a representative of the society or the officers do the collective bargaining for the whole

group. In such an instance, however, a slightly different discipline must prevail in the interests of effectiveness. These two facets of economic bargaining must be clearly understood and clearly delineated in the actions of the society to prevent confusion on the part of members or of users of the service.

In summary, therefore, professionalism, professional standing—however stated—rests upon: (1) an individual, educated, trained and experienced, who is asked to assume responsibilities and exercise judgment in a work requiring special knowledges and skills that serve people's needs; (2) membership of such an individual in a professionally considered society established for the purpose of setting standards and ethics of the working groups as well as planning for constant training and development of its members; and (3) the acceptance of such individuals and societies by the people served as professional persons and professional societies. Professional standing has an importance not related to economics. Such individuals have a definite and important place in the way of life of a community of people. A professional must be accepted and permitted to serve his community of people or he has no standing at all. Economically, communities reward their professional people in accordance with many factors aside from professional standing and that is entirely another phase of discussion.

ABSTRACTS

SEDIMENTATION RATE OF STORED BLOOD, USING SEQUESTRENE AS THE ANTICOAGULANT

The disodium salt of ethylenediamine tetra-acetic acid was used in a concentration of 1 mg. per ml. of blood. Sedimentation was determined by a modified Westergren method.

At room temperature, there was no significant retardation of the sedimentation rate up to 6 hrs. after the blood was taken. Storage for longer periods is often accompanied by a marked retardation.

Storage of sequestrene treated blood in the refrigerator for 24 hrs. does not affect the sedimentation rate, but storage at this temperature for 48 hrs. is usually attended by a marked slowing of the sedimentation rate. Melville, I. D., and Rifkin, B. M., Royal Infirmary, Glasgow, Scotland. *British Medical Journal* Jan. 9, 1960, pp. 107-109.

MALIGNANT CELLS IN A PERIPHERAL BLOOD SMEAR

Reports of malignant cells in a routine blood film have been extremely rare.

In this case report, examination of the blood film revealed a single clump of bizarre cells. This clump consisted of 10 spindle-shaped cells, averaging 20μ in diameter. (A photograph is shown.) The cells were of uniform appearance with numerous adherent platelets. Attention to cytologic detail showed within the nucleus a fine reticular distribution of chromatin with a slight tendency to clump and an indistinct nuclear membrane. Several nuclei contained small nucleoli with a narrow, clear perinucleolar zone. The cytoplasm was moderately abundant and for the most part evenly distributed, with some vacuolization. Their cytologic appearance was unlike that of exfoliated endothelial cells. Finkel, G. C., and Tishkoff, G. H., Medical Research Laboratories of the Yamins Laboratory, Beth Israel Hospital and the Department of Medicine, Harvard Medical School, Boston, Mass. *New England Journal of Medicine* 262:4 1960 pp. 187-188.

MODIFIED KIRCHNER MEDIUM: AN EFFECTIVE AND ECONOMICAL GROWTH MEDIUM FOR MYCOBATERIUM TUBERCULOSIS*

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Introduction

Over the years there have been unceasing efforts on the part of many workers to improve the existing media used for the cultivation of *Mycobacterium tuberculosis*. The greatest share of attention has been given to the perfection of solid media either by modification of established types or by complete innovation. This unremitting search has resulted in the development of a rather large variety of solid media most of which have employed the hen's egg.

In many cases, a solid medium is not the most desirable choice. A liquid medium is indicated if growth on a large scale is needed, and the liquid form is essential if dispersed growth is desired. When a project involves extensive transfer of controlled quantities of tubercle bacilli, a liquid or semi-solid medium offers great convenience in handling.

There have been numerous attempts to produce a good quality liquid medium, but work in this area warrants still further investigation. The earliest significant contribution in the development of a liquid medium for growth of tubercle bacilli was made by Long et al.[6] Other important work was done by Kirchner,[4] Youmans,[10] Dubos[2] and Fisher.[3] The several formulae originated by these men have been modified by many workers in efforts to improve the media.

The material to be detailed in this paper describes still further modifications of existing media, and the advantages resulting from these changes.

The quality of a solid or liquid medium specific for tubercular growth is measured by the degree to which it possesses certain definite characteristics. The medium should: encourage rapid growth of a small number of organisms; allow atypical growth to be easily detected; give a high percentage of positive cultures; be simple to prepare from readily available, inexpensive materials; be selective; and should withstand storage without deterioration.

Kirchner liquid medium, as modified by T. J. Mackie and J. E. McCartney,[8] reportedly satisfied many of these criteria. In addition this medium lent itself well to variation. It was therefore chosen for this study with the idea of adding to its already desirable qualities and to make it less expensive to prepare.

Materials

The basic materials needed to prepare the basic formula for Kirchner medium are as follows:

1. Sodium phosphate (Na_2HPO_4; $12H_2O$)	19 g.
2. Potassium phosphate (KH_2PO_4)	2.5 g.
3. Asparagine	5 g.
4. Sodium citrate ($Na_3C_6H_5O_7$; $2H_2O$)	2.5 g.

* Second Scientific Products Foundation Award in Microbiology 1960. Read before the 28th Annual Convention of ASMT, Atlantic City, New Jersey, June 1960. Received for publication January 1960.

5. Magnesium sulfate ($MgSO_4$; $7H_2O$)	0.6 g.
6. Glycerol	20 ml.
7. Distilled water	1,000 ml.

Number indicates order in which ingredients are dissolved in water. Glycerol is not added until salts are dissolved.

To the Kirchner formula 10% horse serum* and 0.125% Davis agar** were added as suggested by Knox.[5] It was this semi-solid basic formula, now referred to as Kirchner-Davis medium, that variations were made to find an adjunct that would hasten growth with small inoculums, substitute materials that would reduce cost, and additives which would make growth easy to detect. Growth was compared to Youmans' liquid medium,[10] Tarshis and Frisch blood agar,[9] and Lowenstein-Jensen egg medium.[7]

Methods and Results

With the Kirchner-Davis medium growth was detected as early as two days when a heavy inoculum was used. A heavy inoculum was four 3 millimeter loopfuls of a virulent H37Rv strain that was grown for several weeks in basic Kirchner medium, shaken with glass beads prior to use and diluted to approximately a number three barium sulfate nephelometer tube.

Agar Study:

To the Kirchner medium, substitutes for the foreign brand Davis agar were made. Difco agar (Di) and Baltimore Biological Laboratory (BBL) granulated agar were added. The latter two are common bacteriological ingredients found on hand in most American laboratories.

As shown in Table I growth appeared equal to Kirchner-Davis medium when 0.5% of the Difco and BBL agars were used. This percentage of agar was necessary to make the medium of semi-solid consistency essential for proper gas diffusion and the same consistency as that of Davis agar.

TABLE I: AGAR STUDY

Comparison of BBL & Bacto (Difco) agar to Davis agar in Kirchner horse serum medium for growth of M. tuberculosis

Percent of Agar			2 days			7 days			11 days			16 days			21 day		
BBL (B)	Bacto (Di)	Davis (DA)	B	Di	Da	B	Di	Da	B	Di	Da	B	Di	Da	B	Di	D
1.500% (S)	1.500% (S)	-	-	-		-	-		-	-		-	-		-	-	
1.000% (S)	1.000% (S)	-	-	-		-	-		-	-		-	-		-	-	
0.500% (SM)	0.500% (SM)	0.500% (S)	-	-	-	1+	1+	±	2+	2+	1+	2+	2+	2+	4+	4+	3
0.200% (SM)	0.200% (SM)	0.200% (SM)	-	-	1+	1+	1+	1+	2+	2+	3+	2+	2+	3+	4+	4+	4
0.125% (L)	0.125% (L)	0.125% (SM)	-	±	3+	±	±	4+	±	±	4+	1+	1+	4+	2+	2+	4
0.050% (L)	0.050% (L)	0.050% (L)	-	-	2+	-	-	3+	-	-	3+	±	±	3+	1+	1+	4
0.020% (L)	0.020% (L)	0.020% (L)	-	-	-	-	-	2+	-	-	2+	±	±	3+	1+	1+	4

S = Solid medium
SM = Semi-solid medium
L = Liquid medium

* Horse serum (Difco); Difco Laboratories, Detroit, Michigan (6-10 ml. vials @ $3.00).
** Davis bacteriological agar; Davis gelatine (NZ) Ltd., Christchurch, New Zealand (@ $1
per pound).

Drea[1] made a similar study using Long's synthetic liquid medium. He found that tubercular growth was inhibited when 2% agar was added and that 0.1 to 0.25% agar supported growth.

Serum Study:

An additional study was made to find a substitute for the 10% horse serum reported used by Knox.[5] Table II shows the materials used and the growth derived.

TABLE II: SERUM STUDY

Surrogates for horse serum in Kirchner-Davis (0.125% agar) medium for growth of M. tuberculosis

	2 days	7 days	11 days	16 days	21 days
Horse Serum 10%	2+	3+	4+	4+	4+
Bovine Plasma 1%	-	-	-	-	-
Bovine Plasma, Fraction I 1%	-	-	-	-	-
Mucic Acid 1:10,000	-	2+	2+	2+	3+
Gastric Mucin 1:1,000	-	-	-	±	1+
Gastric Mucin 1:10,000	-	1+	2+	3+	4+
PPLO Serum 1%	2+	3+	3+	4+	4+
Purified Human Serum 2%	-	1+	2+	2+	2+
Calf Serum	-	-	1+	2+	3+

- = no growth

± = little growth

4+ = abundant growth

It was found that the most economical substitute was 1% PPLO serum fraction* (an enrichment used for cultivation of pleuropneumonia-like organisms). This material must be added aseptically after the agar base has been autoclaved.

Gastric mucin** in concentration of 1:10,000 and substituted for the serum, supported good growth but it was delayed. This material has an advantage over serum proteins in that it can be added to the medium and autoclaved.

Growth Detection With Use of Dyes:

Water and oil soluble dyes were added to the Kirchner-Davis PPLO medium, with the intention of producing a coloration of growth particles that would be easily detected. Table III indicates materials used.

* PPLO serum fraction: Difco Laboratories, Detroit, Michigan (6-20 ml. vials @ $15.00).
** Gastric mucin: Nutritional Biochemicals Corp., Cleveland, Ohio.

TABLE III: GROWTH DETECTING STUDY

Coloration of M. tuberculosis growth particles when 4+ growth appeared in Kirchner-Davis horse serum medium

1+ = little coloration
4+ = strong coloration

* Oil Soluable Dyes (hot glycerol)	
Sudan III	1+
Sudan IV	2+
Sudan Black B	0
Chlorazol Blue E	0
* Water Soluable Dyes	
Azo Blue	0
Evans Blue	3+
Resazurin	0 (Growth inhibited)
Orange G.	0
Blue Tetrazolium 1:2,000,000	0
Neotetrazolium Phosphate 1:500,000	3+
Neotetrazolium Chloride 1:500,000	4+
Fluorsine Dye ($1:10^{-6}$)	Unsatisfactory, whole medium colored

* All dyes are in concentration of 1:10,000 unless otherwise indicated.

The most favorable and usable color change was found when neotetrazolium salts were used; especially chloride. A concentration of 1:500,000 parts neotetrazolium chloride* was found most ideal. This color reaction when used in the rapid Kirchner-Davis medium would make an ideal culture material for sensitivity studies.

Summary

An effort was made to improve the composition of Kirchner medium as used by Knox[5] for the rapid growth of tubercle bacilli with more economical and readily available materials.

It was found that Difco agar and BBL granulated agar in concentration of 0.5% could be used to prepare a semi-solid medium similar

* Neotetrazolium Chloride: General Biochemicals, Inc., Laboratory Park, Chagrin Falls, Ohio

in growth properties to that obtained when a foreign brand called Davis agar was used. The latter is more desirable, however, for a smaller percentage is necessary to produce the desired consistency of the medium.

Additional savings were made when 1% PPLO serum fraction was substituted for 10% horse serum. Gastric mucin when substituted for serum in a concentration of 1:10,000 supported retarded growth but has an advantage in that it can be autoclaved in the basic medium.

While trying to find a material that would impart color to the growing organism and would make growth easy to detect, it was found that neotetrazolium salts which were colorless in the liquid medium, when reduced by tubercle bacilli produced growth particles that were colored black. This aided in detecting early growth—especially with neotetrazolium chloride in concentration of 1:500,000 and the use of reflected light to read the cultures.

This color reaction in a rapid growth medium would be of great advantage for sensitivity studies. The latter idea is being pursued and will be of value for indirect sensitivity interpretation as shown in Plates I, II and III. A detailed report of this technique is forthcoming.

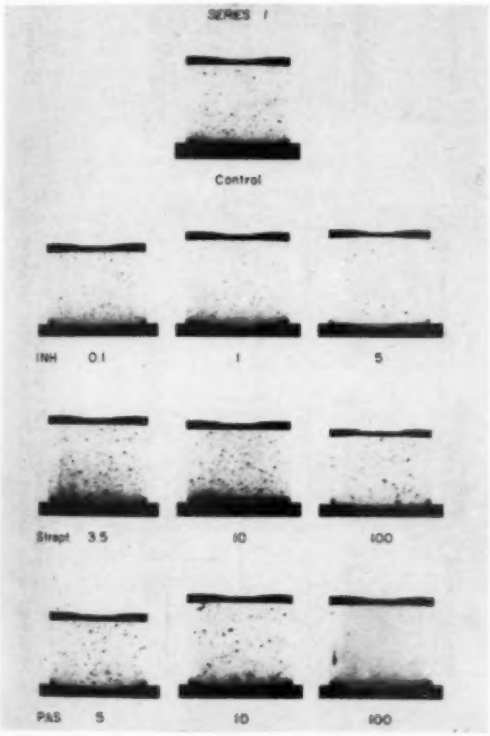

PLATE I.

Sensitivity Study of Tubercle Bacilli
Kirchner Davis Medium with Neotetrazolium Indicator

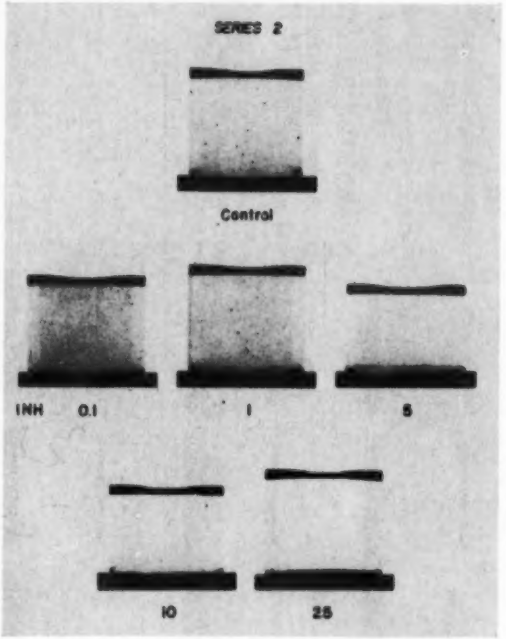

PLATE II.

Sensitivity Study of Tubercle Bacilli
Kirchner Davis Medium with Neotetrazolium Indicator

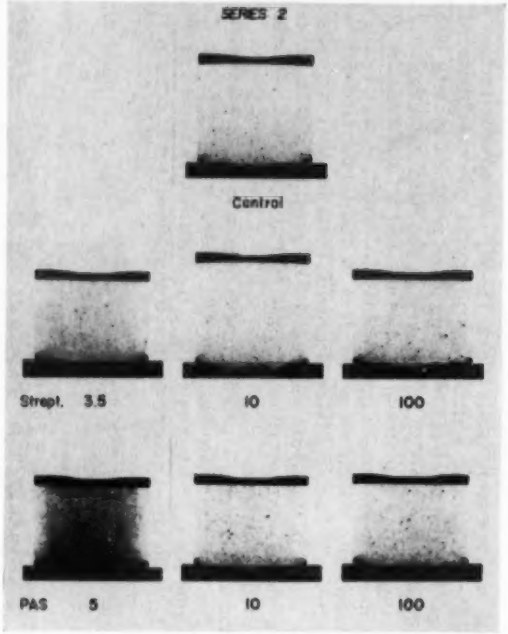

PLATE III.

Sensitivity Study of Tubercle Bacilli
Kirchner Davis Medium with Neotetrazolium Indicator

Acknowledgement

The author wishes to acknowledge the valuable support of Jack Reiss, M.D., Chief, Pulmonary Disease Section, V.A. Hospital, Coral Gables, Florida.

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Sensitivity Study of Tubercle Bacilli
Kirchner Davis Medium with Neotetrazolium Indicator

A MODIFIED TRUE GLUCOSE TECHNIQUE AS COMPARED WITH THE AUTOANALYZER, INCLUDING A MICRO PROCEDURE*

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INTRODUCTION

The use of the Autoanalyzer[4] for blood glucose has created a slight problem in most laboratories that formerly used the Folin-Wu[3] method for blood sugars. Since the Folin-Wu method measures total reducing substances, while the autoanalyzer measures true glucose, the physician might sometimes receive a report with a true glucose value and sometimes one with a total reducing substance on the same patient. This will complicate his treatment of the patient.

While most of the blood sugars are run on the autoanalyzer, a few emergency sugars done at odd hours or on Sundays and holidays are still run by the Folin-Wu technique, because it is impractical to use this instrument for only one sugar. Also when the autoanalyzer is being used for other tests an extreme emergency blood sugar cannot wait for the analyzer to be free, and therefore must be done by the old Folin-Wu method.

Secondly, many laboratories cannot afford the autoanalyzer and must still run their blood sugars by the older methods. Therefore, it was felt that a method other than the Folin-Wu method would be desirable. After trying various techniques, such as the Somogyi$_1$ filtrate plus copper reduction, and the Folin-Wu filtrate plus the Hoffman$_{2,5}$ ferricyanide reagent, true glucose values comparable with the autoanalyzer were not obtained. It was then felt that perhaps a combination of parts of these two methods might possibly work. Therefore a modified Somogyi-Hoffman$_{1,2,5}$ technique was developed. This technique gave true glucose values that compared very favorably with the autoanalyzer.

A micro technique was also developed at the same time so that true glucose values could be obtained on micro amounts of blood. It has not been possible to run micro sugars on the autoanalyzer because of the need of a special dialyzer. This micro technique compares very well with the macro technique as well as with the autoanalyzer. This micro blood sugar can be run on as little as 0.2ml. of blood and therefore finger punctures can be used on small children to obtain this small quantity of blood.

PRINCIPLE

A method was developed using a Somogyi filtrate and Hoffman's potassium ferricyanide reagent. The principle involves the oxidation-reduction reaction of potassium ferricyanide. As in the autoanalyzer technique glucose reduces the yellow color of the alkaline potassium ferricyanide reagent to the colorless ferricyanide compound. Therefore, the higher the glucose value the less the color intensity and the lower the glucose content the greater the color intensity.

*Received for publication January, 1960.

Reagents:

1. 10% solution of zinc sulfate, $ZnSO_4 \cdot 7H_2O$.
2. 0.5 Normal sodium hydroxide.
3. Stock alkaline potassium ferricyanide reagent.
   1.8 gm. potassium ferricyanide
   30 gm. sodium carbonate
   Dilute to 1 liter with distilled water
4. Dilute alkaline potassium ferricyanide reagent.
   Dilute 65 ml. of stock alkaline potassium ferricyanide reagent to 100 ml. with distilled water. Store in a dark bottle.
5. Glucose standards, ranging from 25 to 250 mg./100 ml.

PROCEDURE

Somogyi Filtrate
Macro Technique

Into a chemistry bottle place:

1. 1 ml. oxalated blood
2. 7 ml. distilled water, mix
3. 1 ml. 10% zinc sulfate, mix
4. 1 ml. 0.5N sodium hydroxide, mix and filter.
5. Refilter the first few drops.

Modified Hoffman Method

Into a Folin-Wu sugar tube graduated at the 12.5 mark or in a tube graduated at 16 ml. place:

1. 1 ml. filtrate
2. 2 ml. dilute alkaline potassium ferricyanide reagent
3. Mix and boil 5 minutes in a boiling water bath.
4. Cool in cold water.
5. Dilute to 16 ml. with distilled water or to 12.5 ml. and add 3.5 ml. distilled water.
6. Mix by inversion.
7. Read in a spectrophotometer at wave length 420 mu. against distilled water as a blank.

Somogyi Filtrate
Micro Technique

Into a Wasserman tube place:

1. 0.2 ml. finger blood
2. 3.4 ml. distilled water, mix
3. 0.2 ml. zinc sulfate, mix
4. 0.2 ml. 0.5N sodium hydroxide, mix.
5. Centrifuge for about 5 min.

Modified Hoffman Method

Into a Folin-Wu sugar tube graduated at 12.5 ml. or in a tube graduated at 16 ml. place:

1. 2 ml. supernatant fluid
2. 2 ml. dilute alkaline potassium ferricyanide reagent
3. Mix and boil 5 minutes in a boiling water bath.
4. Cool in cold water.
5. Dilute to 16 ml. with distilled water or to 12.5 ml. and add 3.5 ml. distilled water.
6. Mix by inversion.
7. Read in a spectrophotometer at wave length 420 mu. against distilled water as a blank.

Report in mg./100 ml. blood from a calibration curve prepared from a series of glucose standards ranging from 25 to 250mg./100ml.

As in the autoanalyzer technique glucose values higher than 250 mg.% must be repeated using a dilution of the filtrate and multiplying the final results by the appropriate factor. The normal values for this modified method are the same as that for the autoanalyzer, 60-90 mg.%. A comparison of blood glucose was made on both the autoanalyzer and by this modified Somogyi-Hoffman technique at the same time. The blood used for these techniques was venous blood. The results of both the macro and micro techniques compare very favorably with the autoanalyzer.

Arterial blood was not used for the micro technique since it was desirable to use the same specimen of blood for comparison studies. Therefore, while the micro technique proves just as reliable as the macro technique in its comparison with the autoanalyzer, arterial or finger blood in a non-fasting specimen might result in a slightly higher value than a venous specimen would.

Comparison of Results of a Few Analyses of Blood Glucose by the Autoanalyzer and the Modified Somogyi-Hoffman Macro and Micro Techniques

Sample No.	Autoanalyzer	Modified Somogyi-Hoffman	
		Macro	Micro
1	89 mg. %	90 mg. %	85 mg. %
2	79	80	80
3	64	64	68
4	86	80	85
5	164	160	154
6	42	46	50
7	126	122	130
8	150	146	153
9	130	122	126
10	105	106	106
11	322	324	320
12	156	156	150
13	194	198	209
14	118	112	112
15	56	64	64
16	69	66	66
17	206	195	198
18	205	198	209
19	107	104	100
20	60	52	52
21	133	126	122
22	114	108	118
23	84	76	76
24	200	206	195
25	145	136	140

Comparison of Mean Differences

Autoanalyzer and Macro Technique	Autoanalyzer and Micro Technique	Macro and Micro Techniques
57 cases + 5.2 to — 6.7 mg.	31 cases + 4.8 to — 6.9 mg.	38 cases + 4.0 to — 3.8 mg.

A standard deviation was run on 10 specimens in duplicate by both the autoanalyzer and the modified Somogyi-Hoffman technique.

Autoanalyzer		Modified Somogyi-Hoffman	
1 standard deviation	± 2.27 mg.	1 standard deviation	± 2.45
3 standard deviations	± 6.81 mg.	3 standard deviations	± 7.35

ANAYLSIS OF DATA

The Modified Somogyi-Hoffman technique proves to be reproducible and the difference between its standard deviation and that of the autoanalyzer is insignificant. The mean difference between that of the autoanalyzer and this modified method ranges from + 5.5 to — 6.5 mg. in the macro technique and from + 4.8 to — 6.9 mg. in the micro technique. This difference is no more than the 3 standard deviations value of

± 6.81 mg. of the autoanalyzer itself. The results of the micro method are essentially the same as the macro method or within an allowable error. The mean difference between these two methods was + 4.0 to − 3.8 mg. A total number of 57 specimens was run by both of these methods, the autoanalyzer and the Modified Somogyi-Hoffman technique; of these 49 ranged from 0 to ± 10 in difference, the remainder differed from ± 10 to ± 15. A total number of 31 specimens was run by the autoanalyzer and the micro technique; of these 25 ranged in difference from 0 to ± 10 and the remainder differed from ± 10 to ± 15. A total number of 38 specimens was run by the macro and micro techniques; of these 35 ranged in difference from 0 to ± 10 and the remaining 3 differed from ± 10 to ± 15.

SUMMARY

A macro and micro method for true glucose performed by a modified Somogyi-Hoffman technique was developed and compared with the autoanalyzer. The results of a number of specimens performed by these two methods agree within a value of 3 standard deviations of the autoanalyzer. Also the difference in the standard deviation of each method is insignificant. This modified method utilizes the same principle as the autoanalyzer as well as essentially the same major reagent, an alkaline potassium ferricyanide reagent. The method is easy to perform and takes slightly less time than the popular Folin-Wu method.

This method proves of worth for use during nights, Sundays and other odd times when the autoanalyzer cannot be conveniently run. The values by this method being true glucose will not confuse the physician in his treatment when subsequent specimens are run on the autoanalyzer. The micro method described has a distinct advantage in that true glucose micro sugars can now be run on children, since the autoanalyzer cannot be conveniently run for 1 or 2 micro sugars. Laboratories not possessing an autoanalyzer can now accurately perform true blood glucose by utilizing this technique. In conclusion this modification solves a major problem arising since the use of the autoanalyzer for blood glucose.

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APPENDUM

Since the writing of this paper I have had occasion to determine quantitative urine glucose by this modified Somogyi-Hoffman technique. The results compare very favorably with the autoanalyzer, the greatest difference being no more than 0.1 gm.

Procedure For + to ++ Qualitative Urine Sugar

Into a 100 ml. vol. flask place:

1. 1 ml. urine
2. 50-75 ml. distilled water
3. 1 ml. 10% zinc sulfate, mix
4. 1 ml. 0.5N NaOH, mix
5. Dilute to volume and mix and filter. Urine dilution is 1-100.

Procedure +++ and ++++ Qualitative Urine Sugar

Into a 100 ml. vol. flask place:

1. 0.5 ml. urine
2. 50-75 ml. distilled water
3. 1 ml. 10% zinc sulfate, mix
4. 1 ml. 0.5N NaOH, mix
5. Dilute to volume, mix and filter. Urine dilution is 1-200.

In either dilution use 1 ml. filtrate and 2 ml. dilute alkaline potassium ferricyanide reagent and continue as for blood glucose. Read on the same calibration table as the blood glucose.

Calculation For 1-100 dilution

Multiply mg.% from table by $\frac{10}{1000}$ or divide by 100 for Gm.%.

Calculation For 1-200 dilution

Multiply mg.% from table by $\frac{20}{1000}$ or divide by 50 for Gm.%.

LABORATORY SUGGESTION

USE OF "LESTOIL" IN REMOVING IODINE STAINS*

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It has been found in our laboratory that "Lestoil" (Adell Chemical Company, Holyoke, Mass.) which is described as a solvent soap-sulfonated alcohol type detergent base[1] can be used to easily remove iodine stains from uniforms and other articles. This is done simply by putting undiluted "Lestoil" on the stain, keeping it moist for five to ten minutes and then rinsing with water. The uniform can then be put in a washer and washed in a routine manner without any other treatment. Iodine stains can easily be removed from linoleum and terrazzo floors, in a similar manner without affecting the finish.

This method of removing iodine stains has been found to be better than other methods.

[1] Gleason, et. al., "Clinical Toxicology of Commercial Products," page 645, Williams and Wilkens, Baltimore, 1957.

* Received for publication June 1960.

ABNORMAL HEMOGLOBINS—A REVIEW OF THE LITERATURE*

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The hemoglobins which are designated as abnormal are those in which the globin portion of the molecule differs from the normal form. This type of abnormality is inherited. The abnormal hemoglobins have been studied largely by electrophoresis. Other techniques that have been used for their study include amino acid content, chromatography, solubility, sulfhydryl group content, alkali resistance and immunologic studies.

At a symposium sponsored by the National Institutes of Health in 1953 it was agreed that inherited hemoglobins would be designated by capital letters (Chernoff et al., 1953). Newly discovered inherited hemoglobins are named alphabetically unless there is an outstanding feature which would favor the utilization of another letter. The letter B was omitted from the system. The presence of the hemoglobin previously named B causes red cells to sickle; this species is, therefore, better called hemoglobin S. It was also agreed that if any hemoglobins which were regarded in 1953 as homogeneous were subsequently found to be heterogeneous, the subtypes would be designated by numerical subscripts.

The most common of the inherited hemoglobins are A, F, S and C. Hemoglobin A is the normal adult form. The normal fetal form is hemoglobin F. This species usually disappears at 1 to 2 years of age.

The earliest recorded observation of a hemoglobin abnormality was that made in 1866 by von Körber who noted that the hemoglobins of the adult and of the newborn differ in resistance to alkali (Chernoff, 1958 a). James Herrick is credited with observing, in 1910, sickled cells in the blood of a patient with a severe anemia. This phenomenon of sickling was associated with lack of oxygen in 1927 (Wills, 1957). Probably the greatest single advance in this field was reported in 1949. In that year, the presence in sickle cell anemia of the electrophoretically separable abnormal hemoglobin S was described by Pauling, et al., in an article appropriately entitled "Sickle Cell Anemia: A Molecular Disease" (Pauling, et al., 1949). This investigation was made in the Tiselius moving boundary electrophoresis system. Since 1953, more widespread study of the abnormal hemoglobins has been made possible by the application of the paper electrophoresis technique. According to a review published early in 1959, 26 types of hemoglobin have been reported and their properties studied more or less completely (Beaven, 1959).

A review of the structure of the protein molecule will facilitate understanding the methods by which the abnormal hemoglobins are studied. The primary structure of the protein molecule is a chain of amino acids linked by peptide bonds which join the alpha amino group of one amino acid to the carboxyl group of the next. Thus, additional amino and carboxyl groups and other ionizable groups present in the amino acids are free to ionize under the influence of the medium. The polypeptide chain is folded and coiled in a precise manner for a given protein. This folding

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determines the environment of the constituent amino acids. Folding may also result in steric hindrance of some groups. Unless the peptide chain is cyclic or unless it is bound to a non-protein moiety, there will be present free terminal groups. Since a protein molecule may be composed of more than one peptide chain, there may be more than one mole of free terminal amino acid per mole of protein.

Since proteins are composed of amino acids, an amino acid can serve as a model from which the behavior of a protein in solution can be predicted. In a solution at its isoelectric point, an amino acid exists as a zwitterion or doubly charged ion. When acid is added to such a solution, dissociation of the carboxyl group or groups is repressed and the molecule acquires a net positive charge. Addition of alkali to the solution of an amino acid at its isoelectric point represses the dissociation of the amino group and the molecule becomes negatively charged. Similar dissociations of these groups occur: imidazolium, phenolic hydroxyl, sulfhydryl, and guanidinium groups. The various ionizable groups dissociate at characteristic pH values. They are influenced by environmental factors, such as the proximity of charged groups. The net charge on a protein molecule is the result of the behavior of all of its ionizable groups.

With one qualification to be mentioned later and by some combination of variables, it can be demonstrated that each inherited hemoglobin differs from every other one in its behavior in an electric field. The most important factor determining the electrophoretic mobility of any protein is the net charge on the molecule. This appears to be the only factor which influences the hemoglobins (Itano, 1958). Mobility is influenced by molecular size and shape, but these factors are constant for the hemoglobins which have been studied. Interaction with ions other than the hydrogen ion affects the movement of protein molecules in an electric field, but hemoglobin molecules appear not to undergo such reactions.

The isoelectric point of a protein is that pH at which the protein molecules do not move toward either pole in an electric field. In other words, the net charge on the molecule is 0. The isoelectric point is a characteristic value of a protein. Differences in isoelectric points permit the electrophoretic separation of proteins in a mixture. An isoelectric point is determined by plotting pH against mobility in an electric field. Non-parallel pH-mobility curves indicate that better separation of the mixture can be attained at one pH than at another. Analysis at a pH where all the protein species under study carry a net charge of the same sign avoids or decreases interaction.

The abnormal hemoglobins have been studied by moving boundary electrophoresis and by zone electrophoresis on paper, starch, starch gel and agar. The advantage of the first technique is that absolute mobility can be measured. With the other techniques, mobility must be compared to that of known samples run at the same time. A disadvantage of electrophoresis on paper is that diffusion may obscure a small difference in mobility. Paper electrophoresis can not be done at an acid pH because denaturation occurs. The hemoglobins have been studied as oxyhemoglobin, as carbonmonoxyhemoglobin and, in a few instances, as methemoglobin. The same relative mobility is found for any of these states.

Partial conversion to methemoglobin is to be avoided because it leads to apparent inhomogeneity (Itano, 1957). In cacodylate buffer at pH 6.5, hemoglobin H is negatively charged. These hemoglobins are positively charged and have relative mobilities of this order:

$$C > S = D > G > E > A > F > J > I \text{ (Itano, 1956).}$$

At pH 8.6 in barbital buffer these hemoglobins have a net negative charge and travel in this order:

$$H > I > J > A > F > G > S = D > E > C \text{ (Itano, 1956).}$$

These results were obtained in the Tiselius moving boundary apparatus. Hemoglobins S and D do not separate here or on electrophoresis on paper or starch. The separation of hemoglobins D and S has been reported at pH 6.2 by agar gel electrophoresis (Robinson et al., 1957). Another method, in addition to this one, is needed to establish the presence of hemoglobin D, since it does not separate from A on agar gel electrophoresis. By comparison of their results with those of paper electrophoresis and of chromatography, the authors conclude that their technique combines adsorption and electrophoresis. If this last mentioned technique is considered to be electrophoresis, each inherited hemoglobin differs from every other inherited form in electrophoretic mobility. Separation of a mixture cannot be attained at every pH and with every technique, but by some combination of variables a difference in electrophoretic mobility can be demonstrated.

The chemical basis for the difference in electrophoretic mobility of hemoglobins A, S, C and G has been defined (Hill et al., 1959; Hunt et al., 1959). In one of the two kinds of polypeptide chains present in the hemoglobin molecule, each of the three abnormal forms differs from hemoglobin A in the replacement of one amino acid unit with another. Since the amino acids involved differ in their charge, the result is a difference in net charge on the protein molecule as a whole. Hemoglobins A, S, C and G were digested with trypsin. The resulting peptides were separated in two dimensions by chromatography and electrophoresis. Only one peptide differed in position on the resulting "fingerprints." Abbreviating each amino acid to the first three letters of its name, the composition of that peptide is

Hemoglobin A: val-his-leu-thr-pro-glu-glu-lys
Hemoglobin S: val-his-leu-thr-pro-val-glu-lys
Hemoglobin C: val-his-leu-thr-pro-lys-glu-lys
Hemoglobin G: val-his-leu-thr-pro-glu-gly-lys

The free amino end of the peptide is at the left. Trypsin had hydrolyzed the peptide in hemoglobin C into two shorter units. This peptide is in the beta chain of the hemoglobin molecule; it is felt that it is at the N-terminal end of that chain. It appears that this is the only difference between hemoglobins A and S (Ingram, 1958).

Hemoglobin I has been studied by the same "fingerprint" technique as A, S, C and G (Murayama, 1959). In a peptide different from the key peptide of S, C and G, hemoglobin I contains tryptophan and hemoglobin A does not. Hemoglobin E differs from A in still another peptide (Ingram, 1959). Two forms of hemoglobin D have been described and called D-alpha and D-beta. D-alpha is abnormal in the same peptide as

is hemoglobin I. The abnormality in D-beta is in the peptide which is abnormal in E (Ingram, 1959).

The amino acid content of hemoglobins A and F have been compared (van der Schaaf et al., 1955). These two proteins differ in content of ten amino acids. The outstanding differences are that F has a greater concentration of isoleucine while A has more valine than F. In each mole of hemoglobin A there are five valine residues with free amino groups. Hemoglobin F contains 2.6 N-terminal valine groups per mole of protein (Porter et al., 1948). As is to be expected from the studies mentioned above, it had previously been shown that A, S and C are alike in their N-terminal amino acids.

Hemoglobin A separates into three components on starch block electrophoresis at pH 8.6 (Kunkel et al., 1955). The major form is A_1. A_2 travels more slowly than A_1, with a mobility similar to that of hemoglobin E. A_2 is like A_1 in all other properties studied. Harsh treatment of A_1 did not give rise to A_2. The average normal concentration of A_2 is 2.5% of the total hemoglobin. An increased proportion of A_2 occurs with the thalassemia trait and is felt to be diagnostic (Gerald et al., 1958). A_3 moves faster than A_1 under the conditions described. It appears that A_3 or a material with similar properties can be produced from A_1 (Kunkel, 1958). Studies with radioactive iron are interpreted to mean that A_3 does have some biological significance (Kunkel et al., 1957).

The other inherited hemoglobin which has been divided into subgroups is hemoglobin M (Gerald et al., 1959). The globin portion of hemoglobin M is abnormal. The molecule is also abnormal because it is a methemoglobin and its absorption spectrum in acid solution differs from that of methemoglobin A. The presence of this hemoglobin, therefore, results in cyanosis. Contrary to the convention agreed upon, the subvarieties of hemoglobin M have been designated M_B and M_S to indicate that they were found in Boston and Saskatoon, respectively. These two forms differ from each other and from the normal in their spectral characteristics. The authors who studied these two forms also mention the possible existence of two other subgroups of hemoglobin M.

Chromatographic separation of hemoglobins has been achieved on a weak cation exchange resin at pH 6.3 (Morrison et al., 1957). This separation depends upon the charge on the protein molecule. The most highly charged species is most strongly adsorbed to the column and is eluted last. At pH 6.3, the order of elution of these hemoglobins is F A E S C. Hemoglobin C is eluted from the column last. As is to be expected, this species has the greatest electrophoretic mobility at this pH. This technique, like starch block electrophoresis, separates hemoglobin A into three components.

Studies of the solubility of the hemoglobins are of interest chiefly with regard to hemoglobin S. This hemoglobin is one-hundredth as soluble in the reduced state as in the oxygenated state. This can be compared to hemoglobin A which is one-half as soluble when reduced as when oxygenated. Hemoglobins C and E are twice as soluble as A; D is slightly less soluble than A. A solubility test has been devised in which a mixture of hemoglobins containing no S dissolves completely in the first phosphate buffer used (Itano, 1953). If S is present, the

hemoglobin is studied in a buffer of higher phosphate concentration. This test supplements agar gel electrophoresis in distinguishing hemoglobins S and D; it complements other electrophoretic techniques. This insolubility of hemoglobin S probably explains the formation of sickled cells. A gel of reduced hemoglobin S contains spindle-shaped bodies (Wills, 1957). A sickled cell can be visualized as a bundle of these bodies covered and distorted by a cell membrane.

Additional differences among hemoglobins A, S, C and F are shown by studies of the conformation or spatial arrangement of their sulfhydryl (—SH) groups (Murayama, 1957). These differences may result from differences in folding and coiling the polypeptide chains. The sulfhydryl groups are determined by amperometric titration with silver and mercuric ions. One sulfhydryl group will react with one silver ion. If two sulfhydryl groups are 5.60 Angstroms apart, together they will bind one mercuric ion. Groups farther apart will each react with a mercuric ion. Hemoglobins A, S and C each contain 8 sulfhydryl groups titratable at 38° C.—per mole of protein. However, A has 2, S has 3 and C has no sulfhydryl groups in pairs, 5.60 Angstroms apart. Hemoglobin F is quantitatively different from the others. It contains 6 titratable sulfhydryl groups per mole of protein; these are in three pairs.

Resistance to denaturation by alkali is a characteristic of hemoglobin F and is a tool useful for quantitating this form. Hemoglobin J is reported to be slightly resistant to alkali (Goldberg, 1957); all of the other inherited forms of hemoglobin are labile to alkali. The denaturation of hemoglobin by alkali can be followed by observing the change in absorbance of a solution. Alternatively, the reaction may be stopped, the denatured protein precipitated and the undenatured hemoglobin measured spectrophotometrically (Chernoff, 1958 a).

Absorption of ultra-violet light by hemoglobins A, C, S and F has been studied. Only F differs from the others. Its tryptophan fine structure band is displaced to 2898 Angstroms from the usual position at 2910 Angstroms. Hemoglobins A, S and C have like sedimentation and diffusion constants. There are clear antigenic differences between hemoglobins A and F. As might be expected from the chemical studies, antigenic differences between A and S can be demonstrated only under conditions of maximum precipitation (Wills, 1957).

The abnormal hemoglobins exist in the homozygous state and in the heterzygous state, with the normal form, A, and with other abnormal forms. Genetic studies have shown that the synthesis of hemoglobins A, S, and C is under the control of allelic or closely linked genes (Neel, 1958). An individual who is homozygous for the hemoglobin S gene has sickle cell anemia, a severe hemolytic anemia, and he has sickled cells in vivo. The sickle cell trait, in which hemoglobins S and A are present, is a relatively benign condition; the erythrocytes will sickle under reduced oxygen tension. In this and in most of the other traits, there is present more of hemoglobin A than of the abnormal form. Other heterozygous states have been found. These include S-C, S-D and S-G. Hemoglobin S is the most lethal of the abnormal hemoglobins. Hemoglobin C disease is found in an individual who is homozygous for the hemoglobin C gene. This is a chronic hemolytic anemia. Hemoglobin C trait

is a benign condition. Target cells are found in both of these conditions.

Hemoglobin F production appears to be controlled by a gene at a locus other than the one occupied by the gene for synthesis of A, S and C (Neel, 1958). It appears that the expression of this gene is almost completely suppressed by the homozygous presence of the gene for hemoglobin A, but that the abnormal genes do not suppress the F gene so efficiently. It also seems that hemoglobin F is produced in response to anoxia. Hemoglobin F is found both in individuals who are homozygous and those who are heterozygous for the abnormal hemoglobin genes, in leukemia, in acquired anemias, and in thalassemia. This last disease is not caused by the presence of hemoglobin F or of any other inherited hemoglobin. It is hereditary and it has been found in individuals who also synthesize an abnormal hemoglobin. The term hemoglobin F might better be replaced by the term alkali resistant hemoglobin. As much as 2% of the hemoglobin of a normal adult may be resistant to alkali. On the basis of amino acid content, this hemoglobin appears to be identical with hemoglobin F found in umbilical cord blood (Huisman, 1955). Immunologic studies indicate that the alkali resistant hemoglobin in sickle cell anemia and in other anemias is hemoglobin F (Itano 1956). Determination of the rate of denaturation of the hemoglobin in the same diseases indicates that this is not hemoglobin F (Singer et al. 1951 b).

The genetic control of synthesis of the other abnormal hemoglobins has not been so well studied because they are not so prevalent as are A, S and C. Each of the hemoglobins D, E, G, I and J appears to be controlled by a single gene (Neel, 1958). G, K and P apparently are controlled by a gene or genes at a locus or loci other than that controlling A, S and C (Neel, 1958; Dherte, 1959). Some of the less prevalent abnormal forms have been found in the homozygous state. Like S and C, these are also found with A and with other abnormal forms. Itano published the statement in 1956 that an increased rate of hemolysis is found in all of the homozygous and the doubly abnormal heterozygous states except for the homozygous state for hemoglobin G (Itano et al., 1956). The most recently recognized form at that time was K. It is necessary to study the family in order to establish the homozygosity of an abnormal hemoglobin. A case is reported in which the thalassemia gene suppressed the synthesis of hemoglobin A and only S and F were found (Lehmann, 1957). The thalassemia gene also influences the gene which controls the production of hemoglobin H. It appears that H is formed only in the presence of the thalassemia gene (Lehmann, 1957). Inclusion bodies are seen in erythrocytes containing hemoglobin H (Vella et al., 1958).

A geographic or racial distribution can be described for some of the abnormal hemoglobins. The highest incidence of hemoglobin S is in Africa south of the Sahara. This species is also found in India, at points between Africa and India and of course in the American Negro. The high incidence of S in Africa is explained at least in part by the resistance to malaria associated with the sickle cell trait. Hemoglobin C is most prevalent in West Africa. Hemoglobin E is found predominantly in Indonesia, India and Indochina. Hemoglobin H is most often seen in

the Orient. Hemoglobin J is found in Africa (Zuelzer, 1957). Hemoglobin D is most prevalent among Algerian Moslems and Indians of North Central India (Chernoff, 1958 b).

The minimum standards for defining a new hemoglobin include four types of study (Lehmann, 1957). Electrophoresis or chromatography must be carried out at more than one pH. The solubility of the new hemoglobin should be determined and its absorption spectrum examined. Denaturation by alkali must be studied.

SUMMARY

In summary, the outstanding properties of some of the hemoglobins and a chart (Figure I) of methods for separating and distinguishing them are presented. By some combination of variables, it can be demonstrated that each inherited hemoglobin differs from every other one in its behavior in an electric field. Hemoglobins S and D have been separated only by agar gel electrophoresis, but they can be distinguished by the insolubility of S in the reduced state. Erythrocytes containing

J	I	H	G	F	E	D	C	A	
m 8.6 p 7.8 m 6.5 sol p 8.6	m 8.6 m 6.5 sol p 8.6	m 8.6 m 6.5 sol p 8.6	m 8.6 sol p 8.6	m 8.6 a.d. m 6.5 c 6.3 ag p 8.6 sol	m 8.6 p 8.6 m 6.5 sol c 6.3	ag sol	m 8.6 c 6.3 m 6.5 p 8.6 ag p 7.8 sol	m 8.6 p 7.8 m 6.5 p 8.6 ag c 6.3 sol	S
	m 8.6 m 6.5 p 8.6	m 8.6 m 6.5 p 8.6	m 8.6 m 6.5	m 8.6 m 6.5	m 8.6 m 6.5 p 8.6	m 8.6 m 6.5 p 8.6	m 8.6 m 6.5 p 8.6 p 7.8	m 8.6 m 6.5 p 7.8	J
		m 8.6 m 6.5	m 8.6 m 6.5 p 8.6	m 8.6 m 6.5 a.d. p 8.6	m 8.6 m 6.5 p 8.6	m 8.6 m 6.5 p 8.6	m 8.6 m 6.5 p 8.6	m 8.6 m 6.5 p 8.6	I
			m 8.6 m 6.5 p 8.6	m 8.6 m 6.5 a.d. p 8.6	m 8.6 m 6.5 p 8.6	m 8.6 m 6.5 p 8.6	m 8.6 m 6.5 p 8.6	m 8.6 m 6.5 p 8.6	H
				m 8.6 m 6.5 a.d.	m 8.6 m 6.5 p 8.6	m 8.6 p 8.6	m 8.6 m 6.5 p 8.6	m 8.6 m 6.5	G
					m 8.6 p 8.6 m 6.5 a.d. c 6.3	m 8.6 p 8.6 m 6.5 ag a.d.	m 8.6 c 6.3 m 6.5 p 8.6 ag a.d.	m 8.6 c 6.3 m 6.5 ag a.d.	F
						m 8.6 m 6.5 p 8.6	m 8.6 m 6.5 c 6.3 p 8.6	m 8.6 m 6.5 c 6.3 p 8.6	E
							m 8.6 m 6.5 ag p 8.6	m 8.6 m 6.5 p 8.6	D
								m 8.6 p 8.6 m 6.5 p 7.8 ag c 6.3	C

METHODS OF SEPARATION AND IDENTIFICATION OF ABNORMAL HEMOGLOBINS

m 8.6 — moving boundary electrophoresis, pH 8.6 (Itano, 1956)
m 6.5 — moving boundary electrophoresis, pH 6.5 (Itano, 1956)
ag — agar gel electrophoresis, pH 6.2 (Robinson et al., 1957)
sol — solubility of reduced hemoglobin (Itano, 1953) and sickling of erythrocytes (Hamm, 1952)
a.d. — alkali denaturation (Singer et al., 1951 a)
c 6.3 — chromatography, pH 6.3. (Morrison et al., 1957)
p 8.6 — paper electrophoresis, pH 8.6 (Goldberg, 1957)
p 7.8 — paper electrophoresis, pH 7.8 (Goldberg, 1957)

Figure I

hemoglobin S will sickle on exposure to reduced oxygen tension. Hemoglobin F is resistant to denaturation by alkali. Hemoglobin H is labile to freezing.

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LABORATORY SUGGESTION

THE USE OF "SORBIC"* IN MAINTAINING A CLEAR, CLEAN WATERBATH**

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"Sorbic" (sorbic acid) is a white crystalline powder, slightly soluble in water, which has been found to inhibit yeasts, molds, and other microorganisms.

In this laboratory "Sorbic" has been used with success in maintaining a clear, clean prothrombin waterbath by inhibiting the growth of molds.

The method of use is to take approximately 1-2 grams of "Sorbic" for every gallon of distilled water.

The "Sorbic" does not have to be dissolved before putting in the distilled water as the "Sorbic" eventually goes into solution because of the action of the constant temperature stirrer.

"Sorbic" was also tried in mycological and bacteriological media as well as in the 1% starch solution for amylase but it did not seem to work well for these purposes.

The use of "Sorbic" in waterbaths has been presented which is a simple, economical way of maintaining a clear, clean waterbath.

*Union Carbide Chemicals Company, N.Y. 17, N.Y.

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MICRO-IMMUNOELECTROPHORESIS

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The technique of micro-immunoelectrophoresis, as originally communicated by Scheidegger (1955) has proved extremely useful for the analysis of small quantities of immunologic reactants. The type of electrophoretic cell employed has usually been hand made according to individual laboratory designs. We wish to report the application of the Spinco Model R Paper Electrophoresis System[1] to the micro-immunoelectrophoretic procedure described by Scheidegger.

The Spinco electrophoretic cell is used without load rack or support stand. The cell vessel, with baffles in place, is filled with one liter of buffer. Agar coated slides, as prepared by Scheidegger, are placed across the tops of the wick supports at right angles to the long axis of the cell. Each cell holds 10 slides. Moistened filter paper strips, the width of a slide and of equal length, are used to make contact between the agar of the slide and the buffer of the cell. The materials to be electrophoresed are added to the slides, the cover is placed on the cell vessel, and the cover slot is sealed with tape. Following a 15 minute period of liquid vapor equilibration electrophoresis is begun. Voltage and current are controlled with the RD-2 Duostat power supply.

This technique is both simple and convenient in that it makes use of equipment generally available in the laboratory.

This investigation supported by research grant E-2298 from the National Institute of Allergy and Infectious Diseases, United States Public Health Service.

[1] Beckman Instruments, Inc., Palo Alto, California.

[2] Scheidegger, J. J.: Une micro-methode de l'immuno-electrophorese. Internat. Arch. Allergy, 7, 103-110, 1955.

AMONG THE NEW BOOKS

RECOMMENDED PROCEDURES FOR THE IDENTIFICATION OF SELECTED PATHOGENIC MICROORGANISMS. J. M. Leise and others, U. S. Army Chemical Corps. Revised Nov. 1958, 105 pages. (Order PB 161063 from OTS, U. S. Department of Commerce, Washington 25, D. C., $2.50.

Written for bacteriologists in military or civilian laboratories to whom samples suspected of containing bacteriological warfare agents might be sent for positive identification. Detailed descriptions of methods are grouped together in one section to include rapid identification procedures. The book contains sections on Treatment of Unknown Samples, Initial Identification Procedures, Inoculation of Media, Colonial Descriptions of Pathogenic Microorganisms, Identification, Specific Microorganism Identification, Determination of Antibiotic Susceptibility, Methods, and Materials.

BASIC CLINICAL PARASITOLOGY. David L. Belding, M.D., Professor of Bacteriology and Experimental Pathology, Emeritus, Boston University School of Medicine. Appleton-Century-Crofts, Inc., New York, 1958, 477 pages, 118 figures, 41 tables, $9.00.

A familiar name in parasitology—Belding—has a more detailed approach to the subject, and more from the point of view of a textbook for medical or medical technology student. Frequent use of charts and tables take the place of more wordy descriptive material. There is a lengthy chapter on technical diagnostic methods, as well as one on treatment. More than one volume on this subject could well "round out" the laboratory library.